The Citrus industry / edited by Walter Reuther, Herbert John Webber [and] Leon Dexter Batchelor with the collaboration of J. Henry Burke [and others].

[Berkeley] : University of California, Division of Agricultural Sciences, 1967-1989.

https://hdl.handle.net/2027/pst.000005597284

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THE CITRUS INDUSTRY

VOLUME II

Anatomy, Physiology, Genetics, and Reproduction

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A Centennial Publication of the University of California

Revised Edition
University of California
Division of Agricultural Sciences
1968
Library of Congress Catalog Card No. 67-63041
Printed in the United States of America

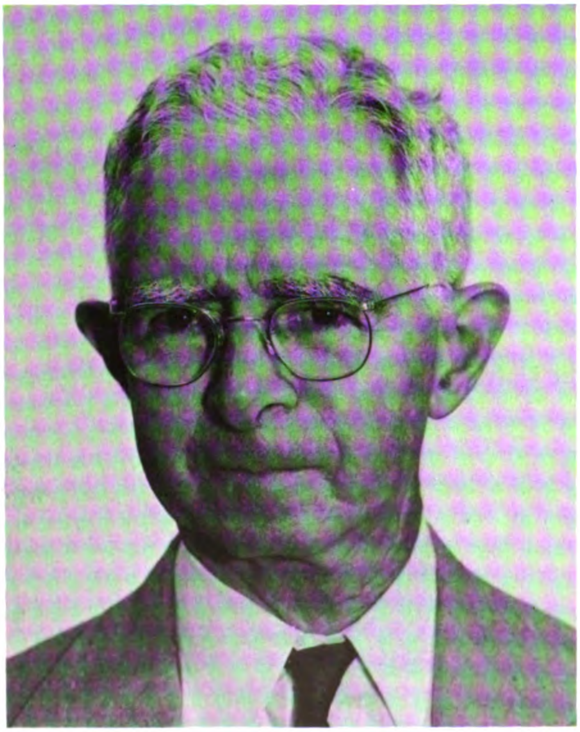
DEDICATED TO

ROBERT WILLARD HODGSON

and

HOWARD BRETT FROST

both of whom possessed the ability to look far ahead and whose talents in divergent ways were reflected in substantial achievements for the citrus industry.



ROBERT WILLARD HODGSON

(1893-1966)

"The direct influence of his teaching can be measured by the enrichment and enhancement of many subtropical fruit industries and the improvement of teaching in agricultural colleges throughout the subtropical regions of the world."

—C. A. Schroeder

The impact of Robert Willard Hodgson—teacher, administrator, researcher, and consultant—on the citrus industries and other subtropical horticulture of the world still cannot be fully assessed. For almost fifty years—until his death on May 17, 1966—Dean Hodgson served as an unofficial horticultural ambassador to numerous citrus-growing nations. Many of Hodgson's former students, representing twenty-eight countries from six continents, are just now beginning to exert agricultural leadership in their homelands. The last product of his voluminous research and writings—a monumental work on citrus varieties of the world—was published posthumously in 1967 in Volume I of the new edition of *The Citrus Industry.*

Somewhat reserved by nature, Hodgson's underlying ardor was always evident when he spoke on the agricultural potential of developing nations. Even the characteristic pipe-tamping ritual that focused the listener's attention on the pipe while Hodgson found time during the pause to judiciously order his thoughts, was less drawn out when he touched upon this subject. He possessed the breadth of vision shared by many of the pioneer citrus investigators—men such as Swingle, Webber, Hume, Coit, and Chandler. Some of his missionary-like proselytyzing for agricultural research and education can probably be traced to the rigid influences of a Methodist upbringing in a clerical family accustomed to

HOWARD BRETT FROST

"Frost's genius lay in a total immersion in research, a gift for meticulous observation, and a rigorous objectivity sustained through decades of citrus experimentation. Few colleagues of the early Citrus Experiment Station days grasped the implications of Frost's solitary and unheralded quest or foresaw the revolutionary impact it would have on today's citrus industry."

—Walton B. Sinclair

When Howard Brett Frost began his citrus studies in 1914 on plantings set out beneath the slopes of Mount Rubidoux in Riverside, California, nucellar embryony in citrus had already been studied by Strasburger and Biermann in Europe and the pioneer hybridizing experiments of Webber and Swingle in Florida had provided a rudimentary framework of citrus genetics. Frost built upon this framework: when he retired in 1948, he left behind a sound structure of knowledge which his colleagues were extending through further basic insights and the development of applications of far-reaching value to citrus horticulture.

The Frost legend persists at the Citrus Research Center and Agricultural Experiment Station in a multitude of stories—both true and apocryphal—about his complete absorption in the subject at hand. During an extended field trip one November, Frost is said to have asked: "What day is it? I promised my wife I'd be home for Thanksgiving." There are anecdotes relating to the thoroughness of Frost's field notebooks, still used by a new generation of geneticists. Riding up the Central Valley with a friend in the 1950's, Frost suddenly produced a notebook enabling him to compare precisely the traveling time from city to city with a trip made twenty years earlier. Such attentiveness to detail made it possible for Frost by persistent and devoted effort to wrest genetic-related information from a plant group in which changes of parish.

Robert Willard Hodgson was born on April 3, 1893, in Dallas, Texas, the son of Mark and Olivia Hodgson. His father, an Englishman, emigrated to America at an early age, entered the ministry, and served in parishes in Texas and New Mexico prior to accepting a post as a supervisor of Methodist church affairs in northern California in 1904. In 1906, after retiring from the ministry, the elder Hodgson purchased an orange grove of questionable varietal value at Thermolita near Oroville.

Mark Hodgson had always been a domineering parent, expecting his six children to mirror his views. His opinions on orange-growing were particularly hard for Robert to accept, conscious as the youth was of his father's mistaken assessment of the orchard. The son's rebellion to parental authority took the form of proving that he had the sounder grasp of agricultural principles. After much study, Robert persuaded his father to let him topwork the trees of variable character to a single variety. The boy's success led to many offers for topworking and propagation from other ranchers, and he was soon helping to manage his father's orchards as well.

Upon entering the University of California at Berkeley, Hodgson inevitably registered in the College of Agriculture. In 1916, he graduated with highest honors in biology and agriculture. He also became the first agricultural student at the University ever elected to Phi Beta Kappa. His talent was recognized by Dr. J. E. Coit who recruited Hodgson as his assistant in citriculture and became a major influence on the student's professional development.

In 1917, Coit was sent to Los Angeles by the College of Agriculture to found the Agricultural Extension Service there and organize the first chapter of the Farm Bureau in southern California. Hodgson, who received his M.S. degree that year, joined Coit as his assistant. Seven years of experience as a farm adviser or "county agent" instilled in Hodgson a lifelong concern for the problems of the farmer, reflected both in the direction of his later research and his approach to education. During this period, Hodgson carried out pioneering research on the adaptation and tolerance of citrus trees and on problems concerned with productivity.

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marker genes and simple inheritance patterns are almost nonexistent.

Howard Brett Frost, descended from French Huguenot and English ancestry, was born on September 28, 1881, in Dairyland, New York. One year prior to his birth, his father George Todd Frost, a former school principal, left teaching because of poor health and purchased a snow-covered farm near the Catskill Mountains. After the spring thaw, it became evident that the terrain was quite rocky. As a child, Frost recalls that "every time we planted new crops our main occupation was picking up stones," which were added to the rock fences between fields. It was on this small farm with its apple orchard that Frost acquired an interest in the growing of fruits.

Frost graduated from Dairyland's elementary school and continued studies on his own until he qualified for a teacher-training course that equipped him to teach children in the one-room schoolhouses of this rural region. Meanwhile, he became interested in several agricultural subjects from articles in *The Rural New Yorker,* a farm magazine. In 1904, after four years of teaching, Frost entered Cornell University, where he registered in the College of Agriculture.

Dr. John Craig, a professor of horticulture who had carried out considerable research on snapdragons, encouraged Frost in plant breeding experiments. In Frost's senior year, he conducted growth studies of Matthiola, the garden stock, and snapdragons in three separate greenhouses, discovering a number of peculiar variations in stocks which he later followed up in graduate work. Frost received his Bachelor of Science degree in 1908. He began graduate school, worked as a graduate assistant at Cornell, and interrupted his studies for about a year to take a Civil Service Commission post in Washington, D.C. He then returned to complete his Master of Science degree, followed by the Ph.D. in 1913.

Frost's work as a graduate student attracted the attention of Dr. Herbert John Webber, head of the department of plant breeding at Cornell, who with Walter T. Swingle had carried out citrus breeding experiments in Florida

In 1924, Hodgson was appointed associate professor of subtropical horticulture at the University of California, Berkeley. In that same year, the Regents adopted a resolution to transfer the Division of Subtropical Horticulture to the Los Angeles campus. When Hodgson was named head of the division in 1925, he immediately became involved in the intensive planning that resulted in the actual move to Los Angeles in 1932. Among his tasks was responsibility for planning and collecting materials for the variety and experimental orchard on the Los Angeles campus, which ultimately became an outstanding facility for research and teaching in the field of subtropical horticulture.

In 1932, Hodgson was promoted to assistant director of the Los Angeles section of the College of Agriculture. In 1935, he received the title of professor of subtropical horticulture. He became assistant dean in 1943, and advanced to the post of dean of the College of Agriculture at Los Angeles in 1952. He retained this position until retiring as dean emeritus in 1961. In 1965, Hodgson was awarded the honorary Doctor of Laws degree by the University of California, Los Angeles, for his outstanding service to the University and his advancement of agricultural science. In conferring this distinction, Chancellor Franklin D. Murphy of the Los Angeles campus praised Hodgson as "one of the pioneers who helped build UCLA into a great institution."

Hodgson's research on orchard efficiency analysis and pruning practices and characteristics of nucellar clones of lemons in California drew attention to many problems of the citrus industry, resulting in improved clones and better cultural and handling methods. His study of exotic citrus varieties in various parts of the world led to numerous introductions of value in breeding, rootstock-scion studies, and other applications. He also stimulated improvement of the avocado and had an influence on the walnut, date, persimmon, pomegranate, and fig industries of California.

Because of his abilities in organization and administration and his skill in appraising problems, Hodgson was sought frequently as a consultant in the fields of agricultural research and education. He was a participant in the United States Technical Assistance Program in Chile in 1957; he was a representative under sponsorship

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from 1892 to 1897. In late 1912, Webber accepted the post of first director of the University of California Citrus Experiment station (now the Citrus Research Center and Agricultural Experiment Station) at Riverside, California. On Webber's recommendation, the Regents of the University hired Frost as a plant breeder. The young scientist followed Webber to California, arriving at the Mount Rubidoux quarters of the experiment station in October, 1913.

In embarking on a career in citrus breeding and genetics, Frost was aware that long years of perseverance would be needed to secure meaningful results. Unlike fruit flies that complete a life cycle in three weeks or tomatoes which require four or five months, the period from seed to fruiting in citrus ranges from five to ten years. This protractedness is compounded by the scarcity of sexually-produced plants obtainable from the seed of many citrus species. Requisite to research that might extend over several generations of trees was a high degree of patience and dedication. Frost had chosen a harsh, lonely outpost of research, and more than three decades were to elapse before the enduring significance of his genetic studies was widely recognized and he was to see some of his hybrids and nucellar budlines attain commercial importance.

Frost's studies advanced the knowledge of genetic structure and crossing behavior in citrus. He was the first to report accurately the normal chromosome number of citrus, he was one of the discoverers of polyploidy in citrus, and was the first American to describe citrus tetraploids. Following up W. T. Swingle's discovery that young trees originating as nucellar-seedling lines from old citrus varieties displayed juvenile characters and thus differed from their parent lines, Frost and others, including pathologists, carried out studies showing that both temporary juvenility and elimination of disease viruses in seed reproduction were involved, as well as occasional genetic variation. Such basic scientific knowledge enabled Frost and his colleagues to develop commercial applications of great benefit to the citrus industry. Today more than half of all lemon and of The Rockefeller Foundation at the Indian Agricultural Research Institute in 1959, 1960, 1961, and 1962; he held a Food and Agriculture Organization consultantship in Cyprus during 1960; and in 1965 was a consultant to the Ministry of Agriculture in Libya. He spent 1951 to 1952 in Egypt as a Fulbright research scholar, and from 1955 to 1959 served as a member of the Administrative Committee of the Inter-American Institute for Agricultural Sciences at Turrialba, Costa Rica. In 1965, he was a consultant to the Faculty of Agriculture at Khartoum University in the Sudan.

His technical reports on horticultural missions in Tunisia, Morocco, India, Egypt, Chile, Palestine, Sudan, Libya, and Tripolitania resulted in substantial horticultural improvements in many of these nations. Among honors bestowed upon him for such services were the Order of Nichan Iftikhar, conferred by the Bey of Tunisia in 1931; the Order of Ouissam Alouite Cherifien presented by the Sultan of Morocco; and the Croix d'Officier du Merite Agricole of the Republic of France.

On the national and local level in this country, Hodgson was also professionally active in a number of organizations associated with agriculture. From 1960 to 1965, he served as chairman of the Walnut Control Board. He was also a member of the California Orange Administrative Board for many years. He was a recipient of the California Avocado Society Award of Honor in 1940, the Los Angeles County Farm Bureau Annual Award of Merit, and the Wilder Silver Medal Award of the American Pomological Society. He was elected a fellow in the American Association for the Advancement of Science, an active member of the New York Academy of Science, and served as president of the Western Section, American Society for Horticultural Science.

Hodgson's prolific publications record includes numerous papers on subtropical fruits and their culture and on agricultural problems in general, many of which were translated into foreign languages. Shortly before his death, he completed an exhaustive monograph on citrus varieties which appears in Volume I of *The Citrus Industry*. While this work is destined to become a classic of citrus literature, Hodgson's greatest legacy lies in the impress of his vigorous mind and ideas on the lives and careers of students and associates who will shape the role of agriculture in the world of tomorrow.

orange varieties propagated in California are vigorous nucellar selections, some of which were originated by Frost. He produced several high-quality mandarin hybrids, including the Kara and Kinnow. Because of their influence throughout the citrus world, however, Frost's greatest contributions are undoubtedly the two fundamental chapters on citrus genetics and reproduction which he wrote for the first edition of *The Citrus Industry* and the results of his research on nucellar-seedling lines.

Throughout his active career, Frost remained associated with the institution at which he began his studies on citrus. Even after achieving emeritus status, he continued work on citrus and on *Matthiola incana*. In the latter area of research, Frost in collaboration with Margaret Mann Lesley discovered a gene entirely new to genetics that controlled chromosome elongation in *Matthiola*. The Frost-Lesley studies included other significant contributions leading to the present methods for commercial production and selection of double-flowered stocks. For many years, Frost was also interested in Esperanto, a form of international language, and he presented an exhibit on the possibilities of international auxiliary languages at the International Congress of Genetics in 1932. The names of three citrus varieties produced by Frost are taken from Esperanto: Trovita (an orange) means "found"; Frua (a mandarin) means "early"; and Sukega (a grapefruit hybrid) means "very juicy."

Frost's membership in scholarly societies has included the American Genetics Association, the American Association for the Advancement of Science, the American Society for Horticultural Science, and the Genetics Society of America. In 1957, he was honored by the California Lemon Men's Club for his distinguished service to the lemon industry. His highest recognition came in 1966 when he was awarded the Wilder Medal of the American Pomological Society, the oldest and one of the most highly prized awards in American horticulture.

PREFACE

Since each of the four volumes of this revised and new edition of *The Citrus Industry* is complete in itself, the general plan of the work must necessarily be restated in each volume. The first volume of the revised edition, *History, World Distribution, Botany,* and *Varieties,* was published by the University of California Division of Agricultural Sciences in 1967. The present volume, *Anatomy, Physiology, Genetics,* and *Reproduction,* encompasses morphology, anatomy, physiology, mineral nutrition, genetics, breeding, seed reproduction, and growth regulators. The remaining two volumes, *Production Technology* and *Biology and Control of Pests and Diseases,* are in course of preparation; the dates of publication, however, cannot yet be announced.

The first edition of *The Citrus Industry,* published in two volumes, served for more than two decades as the classic reference work on the biology and culture of citrus throughout the world. The first volume, *History, Botany and Breeding,* edited by H. J. Webber and L. D. Batchelor, was published by the University of California Press in 1943, followed by two later reprintings. A larger printing of the second volume, *Production of the Crop,* edited by L. D. Batchelor and H. J. Webber, was published in 1948 by the University of California Press. By the 1960's, it had become apparent that many sections of the original volumes had been rendered obsolete by technological advances and the acquisition of new basic knowledge merely touched upon or not considered in the original edition.

Prompted by Dean A. M. Boyce and other colleagues, I agreed to serve as editor for a new, revised edition. Revision of *The Citrus Industry* was initiated on July 21, 1961, as Citrus Research Center and Agricultural Experiment Station Project 2015. Because of substantial expansion of coverage, it became necessary to divide the subjects originally covered in Volume I of the first edition into two volumes. Thus, the present volume includes much of the subject matter that appeared in the first volume of the original work. At the same time, most chapters in this volume represent new treatments of their subjects, reflecting the broader base of experience and information available to the authors as a result of the increased tempo of citrus research in the decades since World War II.

All of the authors of chapters in this volume are members of the staff of the University of California, Riverside, closely affiliated with the Citrus Research Center and Agricultural Experiment Station at Riverside. Their treatment of subjects therefore tends occasionally to exhibit a regional perspective, although improved communications and transportation in the past few decades has made it possible for citrus researchers to be more cognizant of developments in other countries than for contributors to the original volumes.

This new edition of *The Citrus Industry* is intended to present a comprehensive view of all phases of the industry to a broad readership of researchers, administrators, teachers, students, and knowledgeable growers. An effort was made to present material clearly, yet scientifically, so that it might be understood by an intelligent readership. The editor, however, considered it essential that scientific principles on which various practices are based should be explained. Some parts of this volume, therefore, may present material of a highly technical nature best followed by specialists. Literature reviews for most chapters in the volume were completed with 1966 citations, although some authors were permitted to add significant new material during the proofreading of page proofs.

In the first edition, general morphology, histology, and physiology of citrus were treated in a single chapter. In this volume, anatomy and physiology are presented separately in the first two chapters.

Chapter 1, "The Anatomy of Citrus" by Henry Schneider, provides greatly expanded coverage on anatomy. The author has carried out a number of original studies and developed entirely new illustrative material to present as detailed a treatment as possible of the morphology and developmental anatomy of each plant organ.

Chapter 2, "The General Physiology of Citrus" by Louis C. Erickson, concentrates primarily on a discussion of those factors that influence the physiology and metabolism of leaves and fruit. Special attention is also given to some of the physiological disorders of citrus, including such recent problems as those arising from toxic airborne pollutants.

Chapter 3, "The Mineral Nutrition of Citrus," represents a greatly extended treatment of the topic as originally handled by Homer D. Chapman in the first edition. Chapman's new treatment reflects a critical examination of the vast body of recent literature that has enhanced our understanding of the effects of certain mineral deficiencies and excesses on citrus physiology and fruit quality. In addition, methods of controlling nutrient disorders are outlined briefly.

Chapter 4 represents a revision of Howard B. Frost's original chapter on seed reproduction. Robert K. Soost has incorporated much new knowledge on flower biology, embryo development, cytology, and seed germination into this review of the basic biology of seed reproduction in the genus *Citrus*.

Two chapters in the original edition have been synthesized by James W. Cameron into Chapter 5, "Genetics, Breeding, and Nucellar Embryony." Genetics and breeding and bud variation and selection were covered separately in the first edition. Integration of bud variation into the genetics chapter has produced a more unified treatment of disciplines relating to variety improvement. Cameron has also traced the history and progress of citrus breeding activities beginning with the early work of the U. S. Department of Agriculture and indicated the impact of nucellar embryony research on citrus variety improvement.

In Chapter 6, C. W. Coggins, Jr., and H. Z. Hield examine "Plant-Growth Regulators." Although much remains to be learned about these substances and their role in citrus physiology, the authors review the status of knowledge and research and discuss some of the current applications of plant-growth regulators and their potential for the future.

The editor wishes to express his deep appreciation to the authors of the various chapters for their wholehearted cooperation and patience during preparation of this volume. A number of authors also found time despite their busy schedules to assist in the review of chapters by their colleagues.

Gratitude must also be expressed to those who assisted in reviewing parts of the text dealing with their particular specialties and fields of research. Reviewers were as follows: Chapter 1, Katherine Esau, W. W. Thomson, L. C. Erickson, E. S. Ford, and Shirley C. Tucker; Chapter 2, W. W. Jones, Walton B. Sinclair, and Franklin C. Turrell; Chapter 3, Paul Smith and T. W. Embleton; Chapter 4, James W. Cameron, Henry Schneider, and Philip C. Reece; Chapter 5, J. R. Furr, William C. Cooper, Philip C. Reece, and Robert K. Soost; and Chapter 6, William C. Cooper, L. N. Lewis, L. P. Batjer, and C. H. Hendershott.

The photograph of Robert W. Hodgson for the dedicatory page was provided by Evelyn Hodgson, who was also helpful in reviewing the biographical material on her late husband. C. A. Schroeder assisted in providing information for the biographical section.

The dedicatory photograph of Howard B. Frost was generously furnished by his daughter, Mrs. L. W. Towner. Biographical assistance on Dr. Frost was provided by his son, Robert H. Frost, and his brother, Henry Frost.

I wish also to express my thanks for their assistance on editorial and publication problems to William W. Paul, manager of Agricultural Publications, and Lucy G. Lawrence of his editing staff.

WALTER REUTHER
Riverside, California
July, 1968

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CHAPTER 1

The Anatomy of Citrus

HENRY SCHNEIDER

The mature citrus tree is a living, changing entity. Leafy twigs, roots, and the vascular tissues connecting the two are periodically extended, added to, or replaced. After newly formed tissues reach maturity, processes of aging, modification, and degeneration ensue. In typical subtropical climates, flowering and fruit formation occur annually in most citrus species. In tropical areas, flowering and fruit formation is more or less continual, depending on rainfall distribution and other factors. The anatomical aspects of development and senescence of tissues described in this chapter are based primarily on plant materials collected in subtropical California.

Most modern commercial citrus trees have a single trunk. The main branches usually diverge from the trunk at 60 to 120 cm above the ground. The trunk is cylindrical, except in older trees where ridges may form on the trunk above large roots and below large branches. Such ridges are more often found in the lemon than in other citrus species.

The general branching system of cultivated varieties of citrus gives the top or crown of the tree a more or less spherical shape. The form of trees under cultivation varies somewhat with the pruning method. Orange and grapefruit trees produce a dense growth with numerous small branches, whereas the lemon tree has a more open growth with fewer and larger branches. The larger lateral branches of lemon trees grow in a characteristic, eccentric way that results in a flattened limb. Cambial activity is decidedly greater on the lower side of the branch (geotropic growth); consequently, growth rings are distinctly eccentric. Eccentric growth is exhibited also by the lateral limbs of the orange, grapefruit, and other citrus trees, although not commonly so marked as in the lemon. The ability to heal wounds is much stronger on the lower side of such limbs than on the upper. Geotropic growth is shown most clearly by limbs that are approximately horizontal.

Citrus plants in the seedbed have a single primary or taproot. The two or more taproots often observed on older, uprooted citrus trees result from cutting or breaking the original taproot during transplanting from the seedbed or nursery.

Under the influence of a Mediterranean-type, subtropical climate such as California's, citrus species of commerce (except for the lemon) become dormant in winter, but do not shed their leaves. Axillary buds begin to break during warm spells in January and February, but the new shoots do not actively grow until late February or March. A large proportion of the axillary buds grow, particularly those at the extremities of shoots (fig. 1-1, *C*).[1] The resulting spring-cycle shoots are predominantly flower-bearing and vary in composition (Reece, 1945). They may resemble cymose inflorescences and have flowers and aborted leaves (fig. 1–1, *C*, shoots 3 and 6) or have some fully formed leaves, some aborted leaves, and flowers (fig. 1–1, *C*, shoots 4 and 5). Other shoots are leafy with terminal flowers and a few or many axillary flowers (fig. 1–1, *B*), and still others are sterile, vegetative shoots (fig. 1–1, *A*). Flower-bearing shoots predominate on mature trees, and sterile shoots predominate on young trees.

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[1] For the reader's convenience, all figures are presented together at the end of this chapter. Since all details shown by a particular figure are not discussed in any single section of text, there are repeated references to many of the figures throughout the chapter in the course of discussing various organs and tissues.

Flower-bearing shoots tend to be about eight nodes in length. The internodes of leafy, flower-bearing shoots and of sterile shoots are long and triangular in cross-section, whereas the internodes of shoots with aborted leaves are short, circular in cross-section, and may have fewer than eight internodes. In some instances, especially with lemons, the vegetative nature of flower-bearing shoots may be so modified that they appear to be flower stalks with solitary, terminal flowers. Such shoots are short and round, and their aborted leaves are inconspicuous. Flowering of orange trees is profuse in the spring, but most flowers abort and abscise, as do also rudimentary leaves. Twigs usually die if all of their leaves and fruits abscise.

Cambial activity begins during the spring growth cycle and spreads basipetally in the tree, reaching the trunk about a month after it is initiated beneath the buds (Cameron and Schroeder, 1945; Schneider, 1952).

Root elongation like shoot growth occurs in flushes, and some researchers have suggested that shoot growth triggers root growth (Reed and MacDougal, 1937; Schneider, 1952).

In subtropical citrus-growing regions, the summer and fall growth flushes of oranges differ from the spring flush. Usually no flowers form, larger leaves are produced, shoots are longer, sometimes becoming several feet long, and the number of new shoots that grow is small.

This chapter briefly considers the morphology of each organ and describes its developmental anatomy. Since many published anatomical studies do not describe organs in their entirety, several original studies were made by the author to fill in such gaps. Many anatomical studies have been concerned with the sweet orange (*Citrus sinensis* [L.] Osbeck), and most statements about it apply to all commonly grown citrus species, and vice versa. Unless other species are mentioned, it is implied that the author knows from unpublished research that the descriptions also apply to the sweet orange. Some general anatomical considerations of citrus only incidentally related to ontogeny and normal anatomy are reviewed at the end of this chapter. Since literature on citrus anatomy has been reviewed by Hayward and Long (1942), Ford (1942), Scott, Schroeder, and Turrell (1948), and Bartholomew and Reed (1948), a comprehensive survey was not undertaken for this chapter.

THE SHOOT

Leaves, axillary buds, thorns, flowers, and fruits are produced on the citrus stem. Leaves are arranged spirally around the stem, and, in the sweet orange, after the stem is spiraled three times the eighth leaf is directly above the leaf referred to as *n* in figure 1–1, *A*. In other words, the phyllotaxy is 3/8. Direction of spirality, either to the right or left, is determined by counting leaves acropetally. The direction of spirality is reversed with each growth flush (Schroeder, 1953).

Schroeder (1953) reported that in the University of California orchard at Los Angeles the phyllotactic pattern is 3/8 for the following *Citrus* species: *C. medica* L., *C. limon* (L.) Burm. f., *C. reticulata* Blanco, *C. grandis* (L.) Osbeck, *C. paradisi* Macf., *C. aurantifolia* (Christm.) Swing., *C. sinensis* (L.) Osbeck, *C. aurantium* L., *C. ichangensis* Swing., and *C. hystrix* DC. Schroeder also found the phyllotaxy to be 3/8 for *Poncirus trifoliata* (L.) Raf. and *Fortunella margarita* (Lour.) Swing. On the other hand, Banerji (1952) reported a phyllotaxy of 2/5 for *C. grandis*. At the University of California Citrus Research Center, Riverside, the author has observed the phyllotaxy of *C. grandis* and *C. paradisi* to be 2/5.

An axillary bud covered by several prophylls (bud scales) occurs in the axil of each citrus leaf (fig. 1–2, *B*). An axillary bud consists of an apical meristem with leaf primordia as in the apex of a growing stem. Accessory buds develop in the axils of the prophylls; thus, multiple buds are present in the axils of leaves (fig. 1–2, *B*). Axillary thorns may subtend the buds, occurring opposite the first prophyll as shown in figure 1–3, *F* (West and Barnard, 1935). Thorns occur to the left of the axillary bud if spirality is to the left, and to the right if spirality is to the right (Schroeder, 1953).

Individual growth flushes may be distinguished from each other by short, swollen internodes at the beginnings and end of each flush. Moreover, each flush is at a slight angle to the previous one because each new flush originates from an axillary bud. As the stem increases in diameter, the slight zig-zag pattern is obscured (Schroeder, 1953).

The Stem

The newly forming stem is green and tender, with a prominent ridge extending for several centimeters below the base of each petiole (fig. 1–1, *A*). The ridges cause the young stem to be triangular in cross-section, but this form is not maintained when secondary growth appears. Within the ridge at each node, there is a large median leaf trace and two smaller lateral ones (fig. 1–1, *E*). There are also axillary bud and thorn traces (fig. 1–3, *A–F*). Proceeding basipetally down the vascular cylinder, the median leaf trace passes between a lateral leaf trace and a portion of the axillary bud traces of the eighth leaf below. Thus, the arrangement of traces appears related to the 3/8 phyllotaxy (see leaf trace 5 in fig. 1–3, *A–F*).

Below each leaf, within the stem, the various vascular traces to a leaf and its axillary bud and thorn are in a **U**-shaped configuration as seen in transsection, and in the form of a vertical trough as viewed three dimensionally (fig. 1–3, *F*, trace 13). This **U**-shaped configuration also contains the complex of traces associated with the median trace from the eighth leaf above (fig. 1–3, *A*, primordium 5; and its trace in fig. 1–3, *B–E*). The median leaf trace and its lateral traces diverge from the vascular cylinder at a lower level than do the bud and thorn traces (fig. 1–3, *E*, leaf 13). Each of the leaf traces forms a small cylinder after divergence. The lateral leaf bundles unite with the median bundle in the upper portion of the leaf base to form a single cylinder in the petiole (fig. 1–3, *C* and *D*, leaf 13). Above where the leaf bundles diverge, portions of each of the two remaining arms of the **U** become semicircular as seen in transsection, and these unite end to end to form the cylinder of the axillary bud and thorn (fig. 1–3, *C* and *D*).

The Shoot Apex.—At the tip of the elongating shoot is the domelike apical meristem (fig. 1–2, *A*). The single-layered tunica sheathes the corpus, which consists of what appears to be a mass of randomly dividing cells, but in which Frost and Krug (1942) found evidence of two genetically distinct layers. Leaf primordia occur in a spiral around the apical dome and are, of course, successively younger toward the top of the dome (figs. 1–2, *A*; and 1–3). The primordia form as outgrowths from the apical dome and include both tunica and corpus cells.

Leaf primordia are an integral part of the stem tip, but as leaf blades, petioles, and abscission layers differentiate, the leaves become more clearly defined as appendages. Even so, the continuity of leaf and stem should be kept in mind when considering leaf anatomy on subsequent pages (figs. 1–1, *E*; and 1–2, *A*).

The Stem During Procambial Strand Formation.—Procambial strands arise in conjunction with (1) leaf primordia, (2) axillary buds, and (3) either the thorn or primordial floral parts, if one or the other is present. Each strand is a continuous, discrete entity from within the primordial structure it serves to far down into the provascular cylinder (fig. 1–2, *A*). As shown earlier, the median leaf trace, which develops from a procambial strand, remains distinct for more than eight internodes basipetally. Whether each procambial strand forms (1) simultaneously throughout its length, (2) develops basipetally from newly forming primordium into the vascular cylinder, or (3) differentiates from some point in the stem upward into the newly forming primordial structure has not been investigated with regard to citrus. Xylem elements with annular thickenings may appear in the base of the third youngest leaf primordium (Scott *et al.*, 1948; fig. 1–2, *A*), and from there xylem differentiation progresses both basipetally and acropetally. But apparently maturation occurs acropetally (fig. 1–2, *C* and *D*). Sieve tubes, on the other hand, are initiated and mature acropetally. Thus, several growth and development phenomena occur in the provascular cylinder simultaneously, both basipetally and acropetally.

In cross-section, the provascular cylinder of the elongating stem is more or less triangular, and median traces of three leaves form the triangle corner (fig. 1–3, *B* and *C*). Each of the traces at any particular level in the young stem is in a different developmental stage. Degree of development of each trace depends upon the degree of differentiation of the leaf it serves.

The ground meristem is located between the protoderm and the provascular cylinder; the cortex eventually differentiates from it (fig. 1–4, *A*). Cells of the ground meristem are large compared to those of the adjacent meristems, and oil glands differentiate in the outer part. Surrounding the ground meristem is the protoderm (figs.

1–3, *B*; and 1–4, *A*), which is continuous with the tunica of the apical dome (fig. 1–2, *A*). The protoderm cuticle is a thin, continuous film. Where it covers the depression between adjoining cells, the cuticle is thickened and intrudes between the cells (fig. 1–4, *A*). It apparently is not cutinized at this stage, for it does not stain with Sudan IV and does not fluoresce with light having a peak wavelength of 350 mμ. Cells derived from the protoderm differentiate into the epidermis (fig. 1–4, *B–D*). The epidermis is covered by a thick waxy cuticle and contains stomata (fig. 1–5, *D–G*).

The first protophloem sieve tubes differentiate in the outer portion of the procambial strands after the first protoxylem tracheary elements differentiate at the inner margins (fig. 1–2, *C* and *D*). Additional protophloem sieve tubes develop inward from the first-formed ones, whereas the xylem vessels develop outwardly from the first-formed protoxylem vessels. Meanwhile, the procambium, between the protoxylem and the protophloem, produces additional xylem and phloem mother cells. Once xylem and phloem have developed from a procambial strand, the combined tissues are referred to as a vascular bundle. A bundle may be a trace (leaf, bud, or flower trace) or a combination of these called a trace complex.

The Primary Phloem.—As previously mentioned, the protophloem sieve-tube members differentiate from procambial cells. The sieve-tube members are of the same shape and size as the protophloem parenchyma cells, but differ from them in having clear contents (Schneider, 1955). Sieve plates, which occur on the end walls, are more or less transversely oriented. Companion cells, if present, have not been distinguished from protophloem parenchyma cells.

As the internodes elongate, the protophloem sieve tubes are stretched and collapse, but they are replaced by metaphloem sieve tubes derived from the procambium. While the metaphloem is functional, protophloem fibers form from the protophloem parenchyma cells; they elongate by intrusive growth and then form secondary lignified walls (fig. 1–6, *B* and *C*). The primary phloem of the median leaf traces occurs in several bundles, which are elliptical in cross-section. The bundles are separated from each other by large parenchyma cells (fig. 1–6, *B*). The elliptical shape of the bundles seems to result from periclinal divisions of cells surrounding one or a few central cells (fig. 1–6 *D*). Eventually certain procambial cells differentiate into the parenchyma cells that separate the metaphloem from the metaxylem and its procambial cells (fig. 1–6, *B*).

The Primary Xylem.—As early as the third leaf primordium tracheary elements begin to differentiate on the inner side of the procambial strands (fig. 1–2, *A*). Differentiation begins at the base of the primordium and extends basipetally into the stem and acropetally into the leaf. Additional elements differentiate laterally and abaxially from the first ones (fig. 1–6, *D*). The first-formed tracheary elements in leaf primordia are short, fusiform in shape, and have conspicuous annular thickenings (fig. 1–6, *A*; Scott *et al.*, 1948). Later elements have spiral thickenings, are up to 500 microns long, and have tapered ends that overlap other elements. In still later formed elements, thickenings are scalariform. Parenchyma cells lie between the tracheary elements. As the stem elongates, the first-formed protoxylem elements are stretched vertically and collapse (fig. 1–6, *A*). The procambial derivatives from which the metaxylem differentiates occur in radial rows, with the procambium becoming cambium-like (fig. 1–6, *B*). The metaxylem vessels with scalariform thickenings occur in radially arranged rows, and their end walls are transverse and perforated. Each succeeding vessel in a radial row is larger in cross-section than the previously formed one. Parenchyma cells occur between the radial rows of vessels.

The transition from primary to secondary xylem is not readily discernible. Fewer vessels occur in radial rows in secondary xylem than in metaxylem, and fibers are a dominant part of the secondary xylem.

The Mature Primary Stem.—The transition from primary to secondary growth occurs with the beginning of cambial activity. As mentioned earlier, the metaxylem is formed from a cambium-like procambium (fig. 1–6, *B*) which later takes on the functions of the cambium. Therefore the exact time of onset of secondary-xylem formation is not clear. The primary phloem bundles, however, are clearly delimited from the secondary phloem by large parenchyma cells, and the onset of secondary growth occurs with the beginning of secondary-phloem formation.

At the completion of primary development, the cortex is composed of two distinct regions (fig. 1–4, *B*). The inner cortex is composed of large, highly vacuolated cells with thin layers of cytoplasm and thick walls. The cells in the outer

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cortex are small, thin-walled, and contain thick layers of cyotoplasm with chloroplasts (fig. 1–5, *F*). Cells containing calcium oxalate crystals are especially prominent among cortical cells immediately inside the epidermis. These cells become several times as large as adjacent cortical cells and intrude into the epidermis (fig. 1–5, *F*). As the crystal grows, the cell wall thickens, and eventually the protoplast degenerates.

At completion of primary growth, the epidermis contains unspecialized epidermal cells, stomatal guard cells, and oil-gland cover cells (fig. 1–5, *D–G*). The unspecialized, tabular epidermal cells are irregular in shape as seen in tangential section and for a time retain a capacity to stretch tangentially and to divide to accommodate the increase in stem circumference.

Origin of the Cambium.—As the primary stem grows, it has been noted that the metaxylem-forming portion of the procambium *(metaxylem procambium)* takes on characteristics of the cambium in that it cuts off the metaxylem initials in radially arranged rows. The *metaphloem procambium* is set off from the metaxylem procambium by large parenchyma cells. As metaphloem formation is completed, remnants of the metaphloem procambial cells differentiate into metaphloem cells. The metaxylem procambium, on the other hand, is transformed into the cambium. This is accomplished when secondary-phloem initials are cut off.

Cambial cells which add cells to the vertical system are called fusiform initials, although they are not precisely fusiform in shape. They are relatively small and rectangular as seen in cross-section. In tangential section, they are much elongated, with their radial walls parallel through most of their length; at either end they converge, thus forming a wedge. The radial walls are conspicuously pitted (fig. 1–7, *C*). The tangential walls are more or less long rectangles with triangular ends.

The ray initials and their recent derivatives differentiate into rays cells when the cambium is inactive. In contrast, fusiform initials and their immediate derivatives do not differentiate into xylem or phloem cells during periods of inactivity.

The Secondary Phloem.—The functional or conductive portion of the phloem is a moist, soft, glistening, translucent layer of tissue 1 mm or less thick on the inner side of the bark. In addition to *functional phloem,* there are three other more or less distinct layers. These are the *developing phloem,* the *degenerating phloem,* and the *nonfunctional phloem.*

The *developing phloem* occurs adjacent to the cambium; it is transitory in nature, being present during periods of phloem production. In this tissue, phloem mother cells derived from the phloem initials of the cambial zone are transformed into sieve-tube members, companion cells, parenchyma cells, crystal idioblasts, and phloem fibers (fig. 1–7, *A–D*). The parenchyma cells are formed by transverse divisions of cambiform-phloem mother cells (fig. 1–7, *D*). In the formation of sieve-tube members and companion cells, the phloem mother cell divides by a longitudinal wall to form a long, narrow companion cell and a sieve-tube element (fig. 1–7, *D*). Companion cells are usually in contact with the rays. Sieve plates consisting of several sieve areas occur on one of the two converging radial walls at each end of the sieve element as may be seen in figure 1–7, *D* (Schneider, 1952). Nuclei and slime bodies occur in the differentiating sieve-tube members, but both disintegrate at maturity. The formation of fibers, at least in some instances, occurs during temporary stoppages of cambial activity. Bands of fibers form at the end of the grand period of growth and periodically during the remainder of the season (Schneider, 1952). Because fiber formation is erratic, and since several bands form during the season, these bands cannot be used for detecting annual rings. The oldest portion of the *functional phloem* is estimated to be two or more years old—approximately the same age as the oldest leaves. Dormancy callose is not deposited on sieve areas during the winter.

A ring of *degenerating phloem* occurs on the outer margin of the functional phloem. The first evidence that a sieve tube is beginning to degenerate is the appearance of copious callose deposits on the sieve areas, which may be demonstrated by staining the callose with lacmoid. After callose is deposited, sieve-tube contents undergo a lysis and the walls collapse. In some plants, relatively thick callose deposits are normally present on sieve plates of functional sieve tubes, but in citrus phloem callose is so thin it can only be demonstrated with fluorescent dyes.

The *nonfunctional phloem* occurs extensively in the trunk and older branches and consists of layers of fibers, living parenchyma cells, and the dead, collapsed sieve tubes and companion cells (fig. 1–5, *B*).

The Secondary Xylem.—The light-yellow wood of citrus is diffuse porous; in subtropical climates, at least, annual rings may be distinguished with some degree of certainty (fig. 1–8). In sweet orange, cambial activity begins in the twigs in connection with a new flush of growth and spreads basipetally into the scaffold branches, trunk, and roots. At Riverside, California, there is a grand period of cambial activity in the trunks of mature sweet orange trees lasting from about the first of May until middle July (Schneider, 1952). The xylem produced during this period is composed of rays, vessels, parenchyma cells, fibers, and crystal idioblasts (fig. 1–8; DeVilliers, 1939). The vessels are sheathed by living parenchyma cells; some are paratracheal cells and some are ray cells (figs. 1–7, *A*; and 1–8, *C*).

During the summer, cambial activity is erratic, and tangentially arranged bands of metatracheal parenchyma cells tend to form near or between vessels as shown in figure 1–8, *A* (Webber and Fawcett, 1935; Schnieder, 1952). When growth is terminated in the fall by cold weather, a band of small, terminal parenchyma cells forms, within which there may be a few small vessels (fig. 1–8, *A*). This band of terminal parenchyma cells, along with the differences in the character of early and late formed wood, are useful characteristics for distinguishing annual rings. However, in some specimens, bands of metatracheal parenchyma cells form which closely resemble terminal parenchyma. When such bands of parenchyma are followed by formation of thick rings of xylem resembling early wood, a false ring results.

Vessels normally remain free of tyloses and wound gum. However, they do become filled with wound gum as a result of poisoning and disease (Bitancourt, Fawcett, and Wallace, 1943).

Responses of the Cortex and Nonfunctional Phloem to Secondary Growth.—In response to secondary growth, primary tissues of the bark undergo drastic disruption and some degeneration, but they are not sloughed off (figs. 1–4, *B–D*; and 1–5, *A*; Schneider, 1955). When secondary growth first commences, some cells of the inner cortex collapse and die (fig. 1–4, *C*), while others are stretched tangentially. Parenchyma cells between bundles of primary phloem fibers and the cortical cells outwardly from them are stretched tangentially and divide by anticlinal walls (fig. 1–4, *D*). The cuticle and usually the epidermal walls become broken in such a way that vertical cracks appear on the stem (fig. 1–9, *A* and *B*). Periderm formation begins in the cortical cells under these cracks and spreads to the epidermis (fig. 1–9, *C* and *D*). In older stems, dilation of the cortex and phloem occurs entirely in radially arranged, meristematic sheets called dilation meristems, which extend from the secondary-phloem rays to the phellogen (fig. 1–5, *B*; Schneider, 1955). Parenchyma cells and sclereids differentiate from the derivatives of the dilation meristems (fig. 1–10, *A* and *B*).

The Periderm.—The *periderm* gradually replaces the epidermis after the onset of secondary growth. The periderm is composed of the *phellem* and the *phelloderm* (figs. 1–9, *F*; and 1–10, *C* and *D*), which are derived from the transitory *phellogen* (fig. 1–9, *D* and *E*). The phellem protects the bark from drying out, and is composed of layers of cork cells, parenchyma cells, and phelloids; the latter are cuboidal sclerenchyma cells (fig. 1–9, *D–G*). The phelloderm consists of thin-walled parenchyma cells similar to those of the outer cortex.

Phellogen activity occurs in conjunction with the activity of the dilation meristems (fig. 1–10, *C*), with new periderm formation localizing in vertical strips opposite the dilation meristems. The two meristems appear as a **T** in cross-section. Whether a period of phellogen formation over the entire bark occurs at any time during the season is not known. During each period of activity, a new phellogen is formed from the outer phelloderm parenchyma cells (fig. 1–9, *E*); when phellogen activity ceases, the phellogen cells differentiate into either parenchyma or cork cells.

The Leaf

The citrus leaf is unifoliately compound and pinnately reticulate in venation (fig. 1–11, *A*). Abscission zones occur between the leaflet and the petiole and between the petiole and stem (fig. 1–1, *D*). In most *Citrus* species, the petioles are winged. Grapefruit (*C. paradisi* Macf.) and pummelo (*C. grandis* [L.] Osbeck) petiole wings are large, those of sweet orange are smaller, and petioles of the lemon leaf are without wings. Abscission usually occurs at the abscission zone at the base of the petiole, although after certain types of injury, notably those caused by desiccating winds, separation occurs at the junction of the leaf blade and the petiole (fig. 1–1, *D*). Leaf blades of oranges and lemons are oval to oblong in form, dark green on the upper surface and light yellow-green on the lower surface when mature, and may persist for two seasons or more.

The vascular system of the leaf consists of a prominent midvein that becomes somewhat smaller toward the leaf tip because of the branching off of major and minor lateral veins. Most major lateral veins branch near the leaf margin and form a **Y**, and each branch anastomoses with a branch from another major lateral vein. Thus, large leaf areas become enclosed between major lateral veins (fig. 1–11, *A*). These large areas contain a few medium-sized veins that are branches of the major lateral veins and the midvein. These veins anastomose with one another to form several areas within the large ones. Within the resulting mesophyll areas, further branching, rebranching, and anastomoses of veins occur. The resulting network of veinlets enclose small islets of mesophyll within which the tiny veinlets may terminate blindly (fig. 1–11, *B*). In the Rutaceae, there are seven foliar-venation patterns that are related in a specific way to oil glands. The *Citrus* genus has been classified as the simply correlated venation pattern (Dede, 1962). The oil glands are in the areoles, and vascular bundles do not lie above or below them.

Growth of the Leaf.—The newly formed leaf primordia are cylindrical and curve over the apical dome (fig. 1–2, *A*). In cross-section of a shoot, the youngest primordia are sectioned obliquely because of their curved nature (fig. 1–3, *A*, primordia 2 and 3). As the leaf primordium enlarges, it becomes erect and then gradually reflexes away from the axis.

A layer of meristematic cells (the protoderm) covers the surfaces of the leaf primordia. It is continuous with the tunica and matures into epidermis (figs. 1–2, *A*; and 1–3, *A* and *B*). Cell layers beneath the protoderm are derived from the corpus of the apical meristem and differentiate into procambium and ground meristem. The vascular tissues are derived from the procambium, and the mesophyll from the ground meristem.

Each of the cylindrical primordia differentiates into petiole and leaflet. The wings of the petiole and the opposing halves of the leaflet develop from marginal meristems flanking the primordium (fig. 1–3, *A*). The cylindrical portion of the primordium becomes the midrib. A constriction associated with the leaflet-to-petiole abscission zone forms between the petiole and the lamina of the leaf. Oil glands differentiate in the ground meristem early in the leaf's development. They are especially prominent along the margins and a large one appears at the tip of the leaflet (fig. 1–2, *A*; Scott *et al.*, 1948). Oil glands are more prevalent near the upper surface than near the lower surface of the leaf.

The Epidermis.—The upper epidermis of the mature leaf is composed of tabular parenchyma cells covered by a thick layer of cuticle (fig. 1–12, *A*, *B*, and *D*). Stomata are lacking. Large, calcium-oxalate-containing cells occur in the palisade layer and protrude into the epidermis (fig. 1–12, *A*, *B*, and *D*). Thin-walled epidermal cells covering the oil glands are arranged so that two or three cells occur at the center of the cover and other epidermal cells are concentrically arranged around them (fig. 1–13, *C*).

The lower epidermis is composed of tabular parenchyma cells interspersed with stomata as shown in figures 1–12, *C* and *F* (Turrell, 1947). The structure of the stomata both in leaf and stem is similar (figs. 1–5, *D* and *E*; and 1–12, *C*, *E*, and *F*). and consists of a pore and two guard cells. As seen in paradermal section, the two kidney-shaped guard cells form a typical mouth-like opening. The substomatal chamber results from loosely arranged cells with arms and large air spaces between them. An outer chamber is formed where ridges of cuticle overhang the stomatal opening (figs. 1–5, *D*; and 1–12, *C*). A resinous plug may form in this outer chamber (fig. 1–5 *E*) and prevent water from penetrating the stomatal pore (Turrell, 1947). Epidermal cells covering the oil glands (fig. 1–12, *B*) are arranged like those of the upper epidermis.

The Mesophyll.—The palisade parenchyma cells are cylindrical and tightly packed into two or three layers (fig. 1–12, *A*, *B*, and *D*). In leaves with three layers, the cells of the third layer are shorter than the first and second layers and there is air space between them.

The spongy mesophyll is approximately eight layers thick and contains a large amount of intercellular space (figs. 1–11, *B* and *C*; and 1–12, *A*). The spongy cells are saccate, with protruding arms that are attached to arms of adjacent cells (figs. 1–11, *C*; and 1–12, *A*). Near the lower epidermis, the spongy cells are smaller and arms are absent except on cells above the stomata (fig. 1–12, *E*). Armless cells are nearly spherical and tightly packed. The number of tightly packed cell layers is influenced by growing conditions. The leaves of field-grown trees have several such layers and those of greenhouse trees only one (fig. 1–12, *A*).

Structure of the Veins.—The structure of the vascular bundles of the leaf is similar to that of the leaf traces within the stem (fig. 1–3). Within the leaf the bundles are encased in bundle-sheath cells that somewhat resemble spongy parenchyma cells (figs. 1–11, *B–D*, and 1–12, *A*). Secondary growth occurs in the midvein and major lateral veins, but not in the smaller veinlets. As veinlets branch and rebranch, the number of vessels and sieve tubes is reduced. The vein endings may consist of a single tracheid or a uniserrate strand of elongated parenchyma cells enclosed by the bundle sheath.

Abscission Zones and the Petiole.—Abscission zones occur between the lamina and the petiole and between the petiole and stem (figs. 1–1, *A* and *D*; and 1–13, *A* and *B*; Hodgson, 1918; Scott *et al.*, 1948). In certain respects, the structure of the petiole and of the midvein at the base of the lamina resembles that of a stem. A more or less complete vascular cylinder is encircled by ground tissue resembling the cortex (fig. 1–13, *B*). Tissue layers adjacent to the adaxial epidermis are palisade-like (fig. 1–13, *A*). The abscission zone between lamina and petiole occurs at a deep groove; the cuticle at the depth of the groove is so thin it can only be detected with the fluorescence microscope. Primary phloem fibers do not form in the abscission zone, and xylem fibers are mostly absent. Vessels and sieve tubes tend to follow a zig-zag course through the abscission zone, so that they may be cut transversely or obliquely in longitudinal sections (fig. 1–13, *A*). The spherical parenchyma cells of the cortical region have thick, nonlignified walls with conspicuous pit pairs (Hodgson, 1918).

THE ROOT

The Root System.—When the seed germinates, the primary root is the first organ to appear (see fig. 1–31, *C–E*, p. 85). It is massive, white, and, under some cultural conditions, may be covered with root hairs. The number of protoxylem strands (xylem arcs) in the primary root is typically eight (Hayward and Long, 1942). In deep soil, the primary root, if it has not been severed in transplanting, grows straight downward and becomes the taproot. Secondary lateral roots are of two types: large pioneer roots and bunches of fine fibrous roots (fig. 1–14, *B*).

The pioneer roots are large. By branching and rebranching, they and the primary root form the root system framework. Newly formed pioneer roots are of a larger diameter than newly formed fibrous roots (fig. 1–14, *A*). The number of protoxylem strands (xylem arcs) found in pioneer roots is reduced from the eight found in primary roots to about five. Copious secondary vascular thickening occurs.

The fibrous roots occur in small bunches 20 to 30 cm long on the taproots of young seedlings and on pioneer roots of older trees (fig. 1–14, *B*). Branches from the main root of the fibrous bunch are of a uniform size, that is smaller than the main root; subbranches from the first branches are further reduced and also possess a uniformity in size. With each additional subbranching, the roots become smaller, the diameter of the smallest root being less than 0.5 mm. Elongation growth occurs in flushes, with many of the root tips growing simultaneously. The most recent growth flush is set off from the previous flush by a constriction where previous growth ceased and new growth resumed (fig. 1–14, *C*). The number of protoxylem strands (arcs) decreases from five to three or two at second and third levels of branching. Considerable secondary vascular tissue is formed in the main fibrous roots and little or none in the smaller ones (fig. 1–14, *D* and *E*). The cortex is persistent in fibrous roots. The main root of a bunch of fibrous roots is exceptionally important because all materials going to or from the remainder of the cluster funnel through it.

Contrast in Structure of Stem and Root.—The structure of the primary root differs in many respects from that of the primary stem. Root apices are covered by a rootcap (figs. 1–14, *C*; and 1–15, *B*), while stem apices are not. Stem tips produce leaf primordia and have nodes and internodes. Roots lack both. Branch roots arise by formation of an apical meristem in the pericycle (Hayward and Long, 1942), rather than from resting axillary buds, which form, in turn, directly from the stem apical meristem. Roots tend to grow downward, while stems tend to grow upward.

Anatomical differences also can be seen. Oil glands are absent in the roots. The surface of epidermal cells of roots is covered with mucigel (Schneider and Baines, 1964), while cutin sheathes the surface of the epidermal cells of young stems. Epidermal hairs sometimes found on roots are usually lacking on stems, while stomata found in the epidermis of stems are absent in roots. Roots have a hypodermis (exodermis) and endodermis, two tissues which are lacking in stems of *Citrus*. The cell walls of the epidermis of roots contain constituents (probably wound gum and suberin) not found in the epidermis of stems (Cossman, 1939). The protoxylem and protophloem of roots are arranged on alternating radii, rather than collaterally as in stems.

After secondary growth commences, a periderm may form in the pericycle or endodermis of the root which causes the cortex to be sloughed off, whereas the periderm of the stem forms in the outer cortex and tiny remnants of cortex persist between wedges of dilatation tissue. The root phellem lacks the phelloids and parenchyma cells which are found in the stem phellem.

The Root Apex.—In a detailed, well-illustrated description of the ontogeny of the Valencia-orange primary root tip, Hayward and Long (1942) reported that the root apical meristem contains three histogens arranged according to Hanstein's type III, and they noted that Penzig gave a similar interpretation in a study of the lemon root tip. The histogens include a *plerome*, which gives rise to the stele; the *periblem*, which gives rise to the cortex; and the *dermatogen-calyptrogen*, which gives rise to the epidermis and rootcap. At the apex of primary roots, Hayward and Long were able to distinguish between the plerome and periblem, but not between the periblem and dermatogen-calyptrogen. In fine fibrous roots, which have a simpler structure than primary roots, the author found that histogens may be traced into the root apex (fig. 1–15, *A*). Regardless of whether distinct initial layers exist at the apex, the dermatogen-calyptrogen and the periblem become organized into distinct layers a few cells away from the apex (Hayward and Long, 1942).

At the root apex, the dermatogen-calyptrogen layer functions almost entirely as a calyptrogen layer and gives off acropetal columns of rootcap cells. On the curved part of the root tip, a short distance from the apex, derivatives from the dermatogen-calyptrogen undergo oblique division, and cells in that portion of the cap lack an orderly arrangement (fig. 1–15, *A*). Basipetally, where the root is becoming cylindrical, successive sheets of rootcap cells form simultaneously. The most vigorous dermatogen growth occurs immediately basipetally to where the last rootcap cells are initiated; the divisions are anticlinal and the growth intercalary in nature. This region of meristematic activity is considerably removed from the root apex and is sometimes called the protoderm rather than the dermatogen-calyptrogen. Maturation into epidermal cells occurs basipetally to the protoderm.

The periblem appears to be a distinct entity at the apex, and its initials most of the time form a single layer of large cells between the plerome and the dermatogen-calyptrogen (fig. 1–15, *A*). Basipetally from the apex, the number of cell layers in the periblem increases by periclinal divisions until the number of layers found in the cortex for the particular kind of root (primary, pioneer, or fibrous) is reached. Once the final number of layers is reached, meristematic activity is based entirely on anticlinal divisions. The cortical cells elongate axially.

The plerome is a solid cylinder of cells at the center of the root. The pericycle and vascular tissues are derived from it.

In nongrowing or dormant apices, only the apical part of the apical meristem is perpetuated through dormancy; the proximal extremities of the meristem are matured into primary-root tissues (fig. 1–15, *D*). The cells of the meristem become somewhat spherical and vacuolated.

As fibrous roots become fine by rebranching, the apical meristem of the finest subbranches become determinate (fig. 1–15, *C*). Walls of the apical cells of such roots and the plerome become suberized. The proximal parts of the periblem mature into cortex and the dermatogen-calyptrogen becomes suberized.

Maturation of the Rootcap.—The mature rootcap forms a cone-shaped protective cover over the front and sides of the apical meristem. The older cells of the cap slough off, possibly forming a lubricated surface on the soil particle through which the roots grow.

After derivation from the dermatogen-calyptrogen, the rootcap cells along the sides of the root elongate axially and mature as parenchyma cells without further division. As the root elongates, the cells are either stretched to the breaking point or some become detached from the epidermis by the dissolution of the middle lamella (fig. 1–14, *C*). Those rootcap parenchyma cells at the root tip form columns of cells. Parenchyma cells between the apical column and the layered cells on the side of the root lack an orderly arrangement.

The Region of Elongation and Maturation.—The transition from the apical meristem to the region of elongation and then to the mature primary body proceeds more or less similarly in primary, pioneer, and fibrous roots. The most notable differences are that the primary root and pioneer roots have a "pith," more protoxylem strands (arcs), and more cell layers in the cortex than do the fibrous roots.

Epidermal cells immediately basipetal to the protoderm in the region of elongation increase greatly in length, whereas their radial dimension seems to decrease (fig. 1–16, *D*). Root hairs may form (fig. 1–17, *A*, *C*), but conditions favoring their formation are not clearly understood. Good aeration and a pH of 6 or lower are apparently favorable (Hayward and Long, 1942). In addition to epidermal cells with hairs, several other kinds of mature epidermal cells may occur (fig. 1–17, *C*). Each type occurs in patches. In some cell patches, the outer, dome-shaped, tangential walls become thickened, as do also the outer portion of the anticlinal walls. The latter become wedge-shaped. These thick-walled cells remain alive, and their outer surface is coated with mucigel. The outer wall becomes yellow- to olive-brown because of impregnation by an unknown substance (Hayward and Long, 1942). In other areas, the outer periclinal walls remain thin and collapse or rupture. Broken cells contain mucigel (Schneider and Baines, 1964). Mucigel is a mucilaginous material formed by the plant and bacteria between the root and soil zones (Jenny and Grossenbacher, 1962). Transverse cracks sometimes occur in the epidermis and hypodermis, apparently when elongation of the epidermis does not keep pace with elongation of other root tissues (see fig. 4 of Hayward and Long, 1942). In some areas, secondary hairs form by enlargement of hypodermal cells, and part or all of the epidermal cell walls slough off.

The hypodermis is derived from the outer cell layer of the periblem; the region of maximum meristematic activity giving rise to the hypodermis occurs adjacent to the protoderm. Hypodermal cells usually enlarge to several times the volume of epidermal cells (figs. 1–14, *D–F*; and 1–17). The outer periclinal and outer portion of the anticlinal walls become thickened and impregnated with wound gum that reacts with phloroglucinol and hydrochloric acid, but reacts negatively to the Maule test (Rawlins, 1933), indicating the material is not lignin. The thin inner walls of the hypodermis are suberized.

Cortical cells like the hypodermal cells are derived from the periblem. The meristematic activity giving rise to them is not concentrated in one area as is the meristematic activity giving rise to the epidermis and hypodermis. Cortical cells are axially elongated. The outermost, mature cortical cells differ from the inner ones in that they are smaller and thinner-walled, have more cytoplasm and more conspicuous cytoplasmic organelles, and contain more ergastic substances. The endodermis is the innermost layer of cortex cells, and conspicuous Casparian strips, which are suberized, occur on the anticlinal cell walls in mature roots.

The Primary Vascular Tissues.—The outer layer of plerome cells differentiate into the pericycle, and just inside the pericycle the provascular strands develop. Strands alternating with each other differentiate into primary phloem and primary xylem. The first protophloem sieve-tube elements differentiate in advance of the xylem. They are prominent because they lack stainable contents in contrast to the surrounding cells with dense protoplasm (fig. 1–16, *A*). Recognizable companion cells are absent. The protophloem sieve tubes were referred to as ducts by Hayward and Long (1942), because these workers did not detect the end walls (fig. 1–16, *B*). Later-forming, primary sieve tubes differentiate inwardly from the first-formed ones.

The first protoxylem elements develop in portions of the root behind the region of elongation (fig. 1–17, *B*). The protoxylem elements are found near the pericycle and have reticulate thickenings (fig. 1–17, *B*). Later-formed xylem elements are larger and differentiate inwardly from the protoxylem. In small, triarch, fibrous roots, the entire central portion of the root develops into primary xylem (fig. 1–14, *D* and *E*). In pioneer roots with polyarch xylem, however, the procambial cells in the central portion of the stele mature into pithlike, xylem-parenchyma cells (figs. 1–14, *F*; and 1–16, *C*).

Secondary Thickening.—Secondary growth is extensive in pioneer roots; a small amount of growth occurs in main fibrous roots where they arise from pioneer roots, and none or very little occurs in rebranched, fibrous roots (fig. 1–14, *D* and *E*). In rebranched, fibrous roots, cambium formation and secondary xylem formation occur only on the inner sides of the phloem strands. In roots which continue to enlarge, the cambium differentiates laterally in the pericycle and over the primary xylem ridges and forms a complete cylinder. The secondary xylem and secondary

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phloem of the root appear similar to that of the stem.

The degree to which cortical and epidermal tissues are disturbed by secondary growth is proportional to the amount of secondary growth. In diarch and triarch roots with little or no secondary growth, these tissues remain unchanged during the life of the root. Where secondary growth is slight, dilation of the vascular tissue is accommodated by tangential stretching of some cortical cells and by degeneration and collapse of others. Thus, the cortical layer becomes thinner as the stele becomes larger. With continued radial growth, longitudinal cracks occur in the epidermis, hypodermis, and outer cortical cells (Schneider and Baines, 1964). Periderms form centripetal to the cracks in the hypodermis or outer cortex and may extend laterally a short distance. When greater dilatation occurs, a periderm forms in the pericycle or endodermis. In fibrous roots, cells exterior to the pericyclic periderm remain alive for some time. As the root continues to enlarge, increasing numbers of longitudinal cracks occur in the epidermis, hypodermis, and outer cortex. In pioneer roots, the entire cortex is sloughed off.

The cortex of roots from the orchard frequently contains intercellular and intracellular fungus mycelium. Mycelium-free cells that are adjacent to mycelium in intercellular space or to cells invaded by mycelium appear to be normal and do not react to the nearby fungus.

THE FLOWER

Early Differentiation.—In the sweet orange, following winter or drought dormancy, flowers form on new vegetative growth that arises from dormant buds on older leafy stems (fig. 1–1, *B* and *C*). The dormant axillary vegetative buds begin growing as vegetative shoots. However, the vegetative apical meristem of the elongating shoot is transformed into a terminal flower bud about the time the elongating axillary shoot pushes through the bud scales (Nambu, 1931; West and Barnard, 1935; Randhawa and Dinsa, 1947). Leaves on the shoots develop from primordia that were present in the dormant buds (fig. 1–2, *B*), and stem elongation results from intercalary growth. Axillary flower buds develop later than the terminal flower bud, and at a time when the shoots are about 1 cm long (West and Barnard, 1935). A small cushion of meristematic tissue forms in the axil of the leaf primordium. From this cushion arises the axillary flower bud, a bud scale, and between them a vegetative bud (fig. 1–18, *A* and *B*). Most of the sweet orange flowers fall. Of fruits that set on the Shamouti orange (*C. sinensis* [L.] Osbeck), 50 per cent are from terminal flowers and the remaining 50 per cent from one of several axillary flowers (Zacharia, 1951). On many of the shoots, all flowers fall.

In the transformation of the vegetative apex to a flower bud, the apical vegetative meristematic dome becomes broadened and somewhat flattened to form the floral apical meristem, which gives rise to the receptacle and floral appendages (Nambu, 1931; Abbott, 1935). The peduncle is the stemlike region below the receptacle, and it enlarges as the flower enlarges. Primordia of floral parts are finger-like outgrowths from the floral meristem (fig. 1–19, *C;* and 1–20, *A* and *B*), and they curve over it much as leaf primordia curve over the vegetative apical meristem (fig. 1–2, *A*).

Each of the successive whorls of floral parts form above and inside the preceding whorl; the sepals form first and are united at their base to form a cuplike calyx. When the flower bud is small and immature, the distal ends of the sepals are curved around the other flower parts. Later, the enlarging corolla pushes the sepals outward and protrudes through them (fig. 1–1, *B;* and 1–18, *A* and *B*). The petals are slightly overlapped and held together at their margins by interlocking papillae to form a completely enclosed, dome-shaped cover (fig. 1–19, *A*). The stamen primordia form in a single whorl inside the corolla (fig. 1–18, *C*). The anthers and their four locules of microsporocytes are fairly well formed before the filaments elongate (fig. 1–19, *A* and *D*). Development of the anther and pollen are described in Chapter 4.

The pistil consists of an ovary which is composed of a whorl of about ten carpels, a style, and stigma (figs. 1–18, *D;* and 1–19, *A* and *B*). The carpels originate as curved finger-like primordia around the meristematic axis (figs. 1–19, *C*; and 1–20, *A* and *B*). Along each side of the finger-like primordia, marginal meristems occur which produce lamina-like structures resembling those on leaf primordia (fig. 1–20, *B*). The wings of the lamina grow toward the axis. The abaxial side of each lamina is fused with the abaxial side of a lamina of an adjacent carpel primordium; and the margins of the wings are undiverged from the central axis. The compound undiverged wings of the lamina become the septa. Locules occur on the adaxial side of the primordia. In early stages, the locules of the lemon are chambers open at the top (fig. 1–20, *A* and *B*; Ford, 1942). The cells lining the locule are epidermal in nature and indirectly derived from the tunica. The ovules are in axile placentation; in each locule, they occur in two vertical rows, with one row on either side of the stylar canal opening into the locule (see fig. 1–22, *A–C*, p. 67).

The floral disc occurs between the stamens and carpels (fig. 1–19, *A* and *B*), and originates after the carpels (fig. 1–19, *C*; Ford, 1942).

Structure of the Mature Flower.—The *peduncle* of the sweet orange flower is about 1 cm long and less than a millimeter in diameter at its base (fig. 1–18, *A* and *B*). Distally, near the receptacle, the peduncle broadens to about 1.5 mm. At the proximal end of the peduncles of terminal flowers, there is one abscission zone (fig. 1–1, *B*). Axillary flowers have two abscission zones: one where the peduncle attaches to the stem in the axil of the leaf, and another a few millimeters distally (fig. 1–18, *A* and *B*). In the lower part of the peduncle, the vascular cylinder contains five or more collateral bundles, and small accessory bundles occur in the cortex.

The *receptacle* is the broadened upper end of the peduncle to which the floral parts are attached (fig. 1–19, *A* and *B*). Within the receptacle, whorls of vascular bundles diverge from the central cylinder to the various whorls of floral parts (figs. 1–20, *C–E*; and 1–21, *D*).

The *calyx* is a persistent, cuplike structure, usually with five short projections on the rim of the cup, which are the free portions of the individual sepals (figs. 1–18, *D*; and 1–19, *A* and *B*). There is no clear point of transition from the receptacle to the fleshy calyx cup. Sepals are composed of an abaxial and an adaxial epidermis with an intervening parenchymatous mass, within which are found the vascular bundles, crystal idioblasts, and oil glands near the outer epidermis (figs. 1–19, *A–D;* 1–20, *C* and *D;* and 1–21, *C* and *D*). As in the leaf, stomata are found on the abaxial but not the adaxial surface. Venation is parallel.

The *corolla* is usually composed of five distinct petals which alternate with the sepals. They are rather thick and leathery and noticeably reflexed at maturity. The heavily cutinized surface of the petals gives them a glossy or waxy appearance. Internal tissues of the petals are similar to those of the sepals (figs. 1–19, *A*; and 1–21, *B* and *C*). The vascular bundles are virtually parallel, the central one usually being larger than the others. Oil glands are situated, as in the sepals, just under the abaxial epidermis. The epidermal cells early become papillose, especially those near the petal tips and margins (fig. 1–19, *C*; Ford, 1942). As previously mentioned, the petals overlap and the papillae on their epidermal cells interlock. Opening of the corolla is accompanied by shrinkage and plasmolysis of the papillae (Ford, 1942). The stomata are not numerous and usually slightly sunken below the surrounding cells.

The *stamens* (microsporophylls) are usually from twenty to forty in number, and their white filaments are more or less united at their bases into groups (fig. 1–18, *C*). Each filament has one main vascular bundle terminating in the anther (fig. 1–21, *C*). Epidermal cells of the filament are thin-walled, only lightly cutinized, and cubical in shape; stomata are few. The parenchyma cells, which together with the vascular bundle comprise the internal tissue, are at first isodiametric. Later, some of these cells, particularly those just beneath the epidermis, become somewhat elongated. The anthers, which are yellow, are 4-lobed, with a comparatively large cavity in each lobe (figs. 1–18, *C;* 1–19, *A;* and 1–21, *B*). Within the lobes are microspore mother cells that produce pollen grains enclosed by the tapetum (figs. 1–19, *A;* and 1–21, *B*). Tissue lying between the pollen chamber and epidermis is composed of thin-walled parenchyma cells. The epidermal cells of the anthers are comparatively thin-walled and only slightly cutinized.

The *floral disk* (nectary) occurs just within and above the point of attachment of the stamens (figs. 1–18, *D*; and 1–19, *A* and *B*). In early stages of development, until the ovary reaches a diameter of from 8 to 10 mm, the disk may grow upward around the ovary base, forming a shallow cup (fig. 1–19, *A*, *B*, and *D*) As the ovary enlarges, the rim of the cup is pushed outward, and the structure assumes the shape of a disk. (See fig. 1–30, *E*, p. 83.) Until the time the petals fall, outer portions of the disk actively secrete a watery nectar through the stomata, and therefore the disk has been called the nectary (Fahn, 1952).

The *pistil* was considered earlier and is composed of the *stigma, style,* and *ovary* (fig. 1–18, *D*).

The *stigma* is the spherical structure at the top of the style (fig. 1–18, *D*). The stylar canals open on the stigma's surface, and epidermal cells

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of the stigma give rise to papillose hairs which secrete a sweet, viscous fluid.

The *style* is cylindrical, and within it there are as many slit-shaped, stylar canals as ovary locules (figs. 1–18, *D*; 1–21, *B*; and 1–22, *A* and *B*, p. 67). Where the canals open into the locules, there are filamentous epidermal hairs, some of which are multicellular (see fig. 1–25, *C*, p. 73). Vascular bundles in the style are continuations of ovarian bundles. They extend into the stigma, where they branch and terminate just under its surface. In the style, one set of bundles is a distal extension of the fused marginal and septal bundles of the ovary (fig. 1–19, *B*). These bundles are located between stylar canals, where they may be divided into two parallel bundles (fig. 1–21, *B*). Dorsal bundle extensions into the style are located opposite the canals (Ford, 1942). The ground tissue of the style includes thin-walled, cylindrical parenchyma cells plus a few to many oil glands (fig. 1–19, *A*). The epidermis is composed of approximately isodiametric parenchyma cells and cutinized outer walls. The style and stigma are abscissed from the ovary a few days after petal fall. An abscission layer forms at the juncture of the style and apex of the ovary.

The *ovary* structure is considered in the discussion of fruit structure below.

Venation of the Flower.—At the base of the peduncle of the mature lemon *(C. limon)* and lime *(C. aurantifolia)* flower, the vascular cylinder is composed of several discrete bundles. In the receptacle, successive whorls of vascular traces diverge from the vascular cylinder to the perianth, stamens, and carpels (fig. 1–19). The perianth and stamen traces diverge at almost right angles to the vascular cylinder and run more or less horizontally through the receptacle, where they may branch (fig. 1–20, *C–E*).

The sepal traces diverge first, with one trace deflecting toward the median bundle of each sepal. Where the horizontally oriented sepal traces pass under the margins of adjacent petals, branch traces diverge on either side, each to a lateral vein of a petal (Ford, 1942; Venning, 1947).

With lime, the author observed twice as many bundles diverging from the vascular cylinder as the number of sepals (fig. 1–20, *E*). Large traces (sepal traces) diverge toward the midvein of the sepals; smaller traces in the same whorl, which the author calls *petal-sepal* traces, alternate with them (fig. 1–20, *E*). Where the *sepal* traces pass under the margins of petals, there are branch traces (lateral petal traces) at right angles on both sides of the sepal trace. Each lateral petal trace supplies a bundle at the margin of the petal above it (fig. 1–20, *E*, at LP). The main part of the sepal trace continues to a point below the sepal where branch traces diverge from either side of it. The main part of the bundle supplies the median sepal bundle, and the branch traces supply those lateral sepal bundles lying adjacent to the median sepal bundle (fig. 1–20, *E*). The *petal-sepal* traces (fig. 1–20, *E*) pass under the median petal bundle, where each of them give traces to minor lateral petal bundles. The main part of the trace proceeds onward, branches, and serves marginal bundles of adjacent sepals.

The author found a whorl of median petal traces in lime that diverges directly above the petal-sepal traces. Each trace remains unbranched and serves the median bundle of a petal (fig. 1–20, *D*).

In lemon, Ford and Venning did not observe petal-sepal traces, and they reported that petal traces have lateral branches which supply the marginal bundles of the sepals. In contrast to the author's observations, Venning (1947) reported venation of the lime is like that of the lemon as reported by Ford.

The stamen traces diverge above the petal traces and are about twenty in number. Each trace may remain unbranched (fig. 1–20, *C*) or may branch into two or three bundles, each of which supplies one stamen (fig. 1–21, *D*).

In the ovary (see fig. 1–25, *A*, p. 73), there are: (1) *septal* bundles opposite the septa in the pericarp; each septal bundle is thought to be a compound bundle formed by the fusion of bundles of adjacent carpels; (2) prominent *dorsal* bundles opposite the locules in the pericarp; (3) axial bundles in the axis at the lower half of the ovary; (4) the *ovular* bundles, and then (5) the *marginal* bundles, which diverge from the axial bundles. Normally the axial bundles terminate where the marginal bundles diverge from them, but they may carry on to a second fruit as with the navel orange. From the phylogenetic point of view, the marginal bundles, like the septal bundles, are thought to be compound bundles formed by fusion of bundles of adjacent carpels.

The septal traces diverge from the vascular cylinder immediately above the stamen traces and tend to split and anastomose (see fig. 1–25, *A*, p. 73). The whorl of dorsal bundles diverge from the central axis above and alternate with the septal bundles. Small branch bundles diverge from each dorsal bundle and some of them reunite with it, but the dorsal bundles seldom divide into two bundles of equal size. The receptacular axis, hereafter called the axis, occurs at the center of the ovary and is laterally surrounded by margins of the carpels which are fused to it. The vascular cylinder in the proximal part of the axis is composed of about ten orderly arranged, axial bundles (fig. 1–22, *D*). Distally, the ovular traces diverge from these axial bundles (fig. 1–22, *A* and *B*). Above the ovules, the axial bundles diverge into the septa to become the marginal bundles (figs. 1–22, *C;* 1–25, *A*). At the top of the locules, the septal bundles and marginal bundles join, and distally the fused bundles become stylar bundles (fig. 1–19, *B*).

Venation of the flower in the Aurantiodeae, the orange subfamily, has received attention of workers interested in taxonomy and phylogeny because whorls of accessory bundles may represent vestiges of primitive floral parts (Saunders, 1934; Tillson and Bamford, 1938; Ford, 1942; Venning, 1947). Consideration of vestigial bundles is beyond the scope of this chapter.

THE FRUIT

The fruit of citrus is a type of berry sometimes called a hesperidium. It arises through growth and development of the ovary, and consists of approximately ten united carpels clustered around and joined to the floral axis. Phylogenetically, carpels are considered to be modified leaves oriented vertically, with their margins curved adaxially to join the axis and thus form locules into which seeds and juice sacs grow. For descriptive purposes, fruits of citrus have been artificially divided into regions that are not related to phylogeny and ontogeny. Names applied to these regions have some utility; they also cause confusion (e.g. see the section on the septa and central axis below). Figures 1–22 through 1-31, *E* illustrate various parts of the fruit.

The *pericarp* is that portion of the fruit exterior to the locules (fig. 1–29; and 1–31, *A*). It has been divided into three regions (Ford, 1942, p. 304). The *exocarp* or *flavedo* consists of the outermost tissue layers of the fruit. It is composed of the cuticle-covered epidermis and the compactly arranged parenchyma cells adjacent to it (figs. 1–29, *A;* and 1–30, *A*), and it is derived from the abaxial surface of the carpel primordia (figs. 1–19, *C;* and 1-20, *A* and *B*). The *endocarp* is the inner side of the pericarp and a portion of the locular membrane. It is composed of the inner epidermis and several layers of compactly arranged parenchyma cells adjacent to it (fig. 1-29, *D*). It is derived from the adaxial surfaces of the carpel primordia. The *mesocarp* or *albedo* occurs between the exocarp and endocarp and resembles the spongy mesophyll of the leaf (fig. 1–29, *B* and *C*). When the fruit is ripe, the *peel* may be stripped from the endocarp. The peel consists of the exocarp and all but the inner portion of the mesocarp.

The *septa* are fused walls of adjacent carpels. Facing each locule of each carpel is a locular membrane which is a layer of endocarp-like tissue with the epidermal layer on the face of the locule. Between the two locular membranes is a mesocarp-like tissue (figs. 1–22; 1–23; and 1–30, *A–C*). With some citrus fruits this intervening "mesocarpous" tissue is easily torn so that the fruit segments may be separated. The term *segment* is sometimes applied to the locular contents (the juice sacs and seeds) along with the locular membrane in which they are enclosed.

In the flower, the ovules are present in the two rows along the margins of the carpel walls; i.e., where the margins of the carpels join to the axis. The openings of the stylar canals into the locules are between the vertical rows of ovules (fig. 1–22, *A–C*). In the enlarging Valencia orange fruit, a few ovules are transformed into seeds (figs. 1–22, *D;* 1–26; 1–27; and 1–31, *A–E*). Other ovules do not enlarge, although their seed coats may continue to develop (figs. 1–23, *A*–2; and 1–31, *A*).

Growth of the Valencia orange fruit occurs in three periods or stages (Bain, 1958). *Stage I* lasts for several months after petal fall and is characterized by increase in thickness of the pericarp as a result of cell division (fig. 1–22, *A–D*). *Stage II* lasts for about six months and is characterized by cell enlargement and cell differentiation of the pericarp, and an increase in size of the locules (fig. 1–23, *A*–1 and *A*-2). During *Stage III*, the fruit matures, the color changes, and the growth rate slows (figs. 1–29 and 1–30).

The Juice Sacs (Vesicles)

The juice sacs are large club-shaped structures that grow from the endocarp into the locules and compactly fill them (figs. 1–22; 1–23; 1–24; 1–25, *A;* 1–30; and 1–31, *A*). Some sacs on long stalks reach the central axis, while other sacs on short stalks fill the outer part of the locule. Stalks

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are usually slender and thready but may be somewhat bulky. The mature vesicles are saclike in nature in that they have a tough, skinlike, cuticle-covered epidermis that encloses large vacuolated cells containing juice that is released when the fruit is squeezed (fig. 1–30, *C*; Grew, 1682; Tschirch and Oesterle, 1900; Matlack, 1931). In some fruits, the sacs stick together, and their individual nature is not apparent until they are teased apart (fig. 1–31, *A*). The thin-walled, fragile, polyhedral cells of the sac are about 150 microns in diameter, but clusters of smaller cells with prominent nuclei, plastids, and oil droplets may occur in the central region of the sac (Dodd, 1944). This core of smaller cells with fatty droplets may be the "globular body" described by Davis (1932) from macroscopic observation. His anatomical description of the globular body remains unconfirmed and contains contradictory statements. Outwardly from the large polygonal cells and just inside the epidermis there may be several layers of tabular cells that did not differentiate into large parenchyma cells. Their long axes are at right angles to the axis of the sac and to the axes of the epidermal cells. The epidermal cells are elongated in the direction of the sac axis and are several hundred microns in length, 10 to 20 microns wide, about 20 microns thick, and have a waxy cuticle on their outer side.

The stalks of the sacs have a peripheral layer of cuticle-covered epidermal cells, which are usually much elongated or, in some instances, variously shaped. The internal cells of the stalks are parenchymatous. They may be cylindrical or of various shapes and sizes, with a few calcium oxalate cells interspersed. There are no vascular bundles in the stalks (fig. 1–30, *C*; Bain, 1958; Kordan, 1963). In occasional stalks of grapefruit (*C. paradisi*) and shaddock (*C. grandis*), the author has observed cells with secondary, thickened walls. These are most frequently the elongated epidermal cells (Penzig, 1887); groups of cells or individual cells within the central part of the stalk may also have secondary thickenings (fig. 1–30, *D*). The thick-walled cells may resemble tracheids. In grapefruit, they are about 10 microns in diameter, with a lumen of about 6 microns, and about 500 microns long. The tracheid cells may lie side by side and end to end, with their tapered ends overlapping to form a continuous strand up to two millimeters. The randomly distributed, isolated group of cells do not have continuity with vascular bundles in the pericarp. In addition to the tracheid cells, occasional vessel elements have been observed. Xylem vessels with helical thickenings have been reported in Eureka lemon fruits by Kordan (1964), but it is impossible to determine from his photomicrographs if the end walls are perforated, which would be necessary to confirm that they are vessels. The author made serial, paraffin sections of locules of several mature Valencia orange fruits, but no tracheary elements were observed in the juice sac stalks.

Under the anoptral contrast microscope, streaming of mitochondria may be readily observed in the large juice-sac cells. Thick sections squashed under a coverslip are satisfactory for such observations.

Formation of the Sacs.—Since the juice sacs are the edible portion of the fruit, they are of uppermost economic importance, and their bizarre nature gives them considerable scientific interest. The author has therefore studied their ontogeny in the Valencia orange.

Ovaries in various stages of development were sectioned beginning with those in flowers whose petals had not yet opened (fig. 1–22, *A*). Juice sacs were initiated from about the time the petals opened until after the style fell and were dome-shaped projections into the locules from the endocarp (fig. 1–24). When the domes first appeared, divisions in the epidermis were oblique, anticlinal, and periclinal (fig. 1–24, *A–F*). The daughter cells retained characteristics of meristematic cells. Subepidermal cells also divided, but often their daughter cells enlarged and lost their meristematic characteristics. Cone-shaped cells that fitted into the newly forming dome resulted from the oblique divisions of the epidermal cells and the manner of subsequent cell enlargement (fig. 1–24). The inner daughter cells of obliquely and periclinally divided epidermal cells became subepidermal as cell divisions continued. The meristem that produces juice-sac elongation occurs at the tip of the sac (fig. 1–24, *H*); since cells at the meristem's distal end continue to divide and those at the proximal end enlarge and differentiate into stalk cells, it seems likely that derivatives of any cells derived from the epidermis would quickly become dominant in the meristem. Hence, all cells of the sac could be of epidermal origin. On the other hand, if divisions in some sac primordia were predominantly anticlinal, the inner portion of such sacs would be partly or entirely derived from subepidermal tissue of the endocarp (fig. 1–24, *B*, left).

Determination of the tissue from which juice sacs originate has received considerable attention prior to the author's studies on Valencia orange. In studies of pink-fleshed grapefruit with white pericarp, Cameron, Soost, and Olson (1964) hypothesized that the pink color of juice sacs resulted from a chimeral condition. They postulated that juice sacs are of epidermal origin, and the epidermis carries the genetic factors for redness. The author of this chapter observed that the pink color results from thin, red, platelet-shaped crystals that are located in the cytoplasm and are presumably lycopene. Histological studies by Ford (1942) indicate that the juice sacs of lemon are at least partially of epidermal origin. For the sour orange *(C. aurantium)*, Poulsen (1877) in his drawings shows some periclinal divisions in the epidermis in newly forming juice sacs. Others have indicated that only the epidermis of the sour-orange juice sac is of epidermal origin, and that inner tissues are derived from subepidermal layers of the endocarp (Biermann, 1896; Tschirch and Oesterle, 1900; Hartl, 1957); a similar origin for juice sacs has been reported for *C. grandis* (Banerji, 1954).

Once the juice sac of Valencia orange has been initiated, a period of apical growth ensues during which the slender stalk of the sac is formed (fig. 1–24, *H*). During this and subsequent growth periods, only anticlinal divisions were observed in the epidermis.

After the stalk is formed, the juice sac continues to elongate by apical meristem activity, and an obese mass of meristematic tissue forms subterminally to the apical meristem (fig. 1–23, *C*). The obese meristem gives rise to the body of the sac. Proximally to the obese meristem, there is cell enlargement which begins at the center of the sac and progresses outwardly (fig. 1–23, *A*–2); in the fully developed sac, all of the cells from the center to the epidermis are enlarged (fig. 1–30, *A* and *C*).

The Seed

The polyembryonic seeds are derived from the ovules through a series of growth and developmental changes. Their several embryos are covered by an inner coat that is thin, dry, and brown, and an outer coat (the testa) that is tough, leathery, straw-colored, and slippery when wet (fig. 1–31, *A–E*). The chalazal end of the inner coat is derived from a portion of the chalaza, and it is thicker and darker colored than the sides and micropylar end of the coat, which derive from the inner integument (fig. 1–31, *B* and *D*). As in other dicotyledonous seeds, the radicals are at the micropylar end and the cotyledons at the chalazal end.

The ovules occur in two rows in each carpel: hypothetically, one along each margin of the carpellary leaf; i.e., where the margins of the carpels join to the central axis (fig. 1–22, *A–C*; Ross, 1890). The stylar canals occur near the top of the locules and between and inwardly from the rows of ovules (fig. 1–22, *A–C*).

The radially oriented, slit-shaped stylar canals open into the locules along their centrifugal side. The radial dimension of the canal at the top of the locules is equivalent to that in the style, but it gradually diminishes basipetally and fades out (figs. 1–21, *B*; and 1–22, *A–D*). Where they open into the locules, the stylar canals are lined with epidermal hairs that grow out into the locules and around the micropyles of the ovules (figs. 1–21, *A*; 1–22, *B* and *C*; and 1–25, *B–D*). Perhaps it is these hairs that secrete mucilaginous fluid found in the locules of young ovaries (fig. 1–25, *C* and *D*), but the carpellary emergences with their disintegrating middle lamellae and cell walls very likely contribute some of the gummy material found in the locules (figs. 1–23, *B*; and 1–24, *H*). Emergences are especially abundant at the bottoms of the locules. When gummy materials are abundant in the bottoms of the locules, certain aberrations occur in the development of the elongating juice sacs. Upon dehydration for embedding and sectioning, the amorphous mucilaginous fluid solidifies and becomes brittle and cracked.

The ovules are on a very short funiculus located near the upper end of the cup-shaped ovular wall (figs. 1–21, *A*; and 1–25, *B* and *D*). The upper, outer part of the ovular wall is called the outer integument. The funiculus is attached to one side of it, and the integument at the point of attachment is much thickened and contains a vascular bundle running from the funiculus to the chalaza at the bottom of the cup (fig. 1–25, *B*). In the chalaza, the bundle terminates as a bowl-shaped plexus or cap of vascular tissue (figs. 1–25, *B*; and 1–27, *C* and *D*). From the chalaza grow the integuments and the nucellus (fig. 1–21, *A*). The micropylar end of the ovules is usually oriented upward, and later it is tipped toward the stylar canals (figs. 1–21, *A*; and 1–25, *B*). The opening in the ovule wall (the micropyle) is formed by slit-shaped openings in the integuments, with the slits usually radially oriented (figs. 1–22, *B* and *C*; and 1–25, *B*). Sometimes the slit of the inner integument is turned crosswise to that of the outer integument.

Most ovules of the Valencia orange abort either before a conspicuous embryo sac forms or soon thereafter. Although aborted ovules do not enlarge, their integuments may develop seed coats (figs. 1–23, *A*–2 and *C*; and 1–31, *A*).

In ovules destined to become seeds, the nucellus greatly enlarges, while the endosperm slowly begins to develop (fig. 1–26, *A* and B). The cytology of formation of the megaspore (young embryo sac), the embryo, and the endosperm are presented in Chapter 4. As the seed enlarges, the endosperm becomes an extensive tissue, gradually surpassing the nucellus in volume (fig. 1–26, *C*; and 1–27, *A* and *B*). The nucellus apparently acts as a nurse tissue for the endosperm, and no doubt functions in absorption and translocation of nutritive materials from the variously purple- to red- to brown-colored cells of the chalaza, which in turn absorb them from the vascular tissues in the chalaza. Cells of the nucellus—especially at its base—are elongated in such a way as to be adapted to transport (fig. 1–27, *C*). Because of stretching accompanying seed enlargement and possibly owing to adsorption by the expanding endosperm, the nucellus gradually becomes thin.

Several nucellar embryos become conspicuous in the endosperm, and stalks supporting the embryos are attached to the nucellus (fig. 1–27, *A* and *B*). The enlarging embryos gradually replace most of the endosperm. The quantity of ergastic materials in the mature embryos is far too great to have been obtained purely by adsorption of the endosperm, and the endosperm must act as a conductive tissue, with the seed vascular bundle, the plexus of vascular tissue in the chalaza, the brown layer in the chalaza, and the intervening nucellus figuring very importantly in the transport of food. The cytoplasm of the isodiametric parenchyma cells of the cotyledons is very thick and contains abundant aleurone granules (Winton and Winton, 1932–39).

The micropylar end of the *inner seed coat* is composed of remnants of the endosperm, nucellus, inner integument, and the inner part of the outer integument; the chalazal end is composed of remnants of endosperm, nucellus, and all the tissues of the chalaza except the epidermis and a few adjoining layers of parenchyma cells (figs. 1–27; 1–28; and 1–31). The inner seed coat receives its color from the layer of brown-to-reddish or purple epidermal cells of the inner integument and at the chalazal end from the several layers of like-colored cells in the chalaza adjacent to the nucellus (figs. 1–27; and 1–28, *A*, *B*, and *D*).

In the developing seed, the variously colored inner epidermis of the inner integument (called the "*braune haut*" in the sour orange by Tschirch and Oesterle, 1900) becomes prominent early, because the cells contain a colored vacuolar fluid (figs. 1–26, *D*; and 1–28, *A* and *B*). In the chalaza, the several layers of colored cells are continuous with those of the inner integument (fig. 1–28, *B*). and the nucellus is isolated from the vascular tissues in the chalaza and the integuments by these peculiar cells. The shade of color of the chalaza resulting from the vacuolar material has been used as a taxonomic character; in the blood oranges it is red (Chapot, 1964).

The *outer seed coat* (the testa) is composed primarily of the outer epidermis of the ovular wall. During final stages of seed maturation, the epidermal cells form lignified, secondary walls. The cells are axially elongated with tapered, wedge-shaped ends that interlock with the wedge-shaped ends of other cells (fig. 1–28, *C*). Their outer tangential walls have papillae and are covered with a thick, mucilaginous layer (fig. 1–28, *D* and *E*). The character of the papillae vary from species to species (Winton and Winton, 1932–39). Adhering to the inner surface of the epidermis are thin-walled parenchyma cells of the outer integument, and at the chalazal end of the seed, tissues of the chalaza. These adhering cells eventually die and collapse.

The Pericarp

The Exocarp (Flavedo).—The *exocarp* or outer rind (fig. 1–29, *A*) is composed of tissues resembling those of the outer part of the primary stem (fig. 1–5, *F*) and of the abaxial surface of the leaf (fig. 1–12, *A*–*C*; Scott and Baker, 1947). The epidermis is composed of tabular parenchyma cells, guard cells, accessory cells, and oil-gland-cover cells. A thick cuticle covers the surface and intrudes part way between the anticlinal walls of cells (fig. 1–29, *A*). Scott and Baker (1947) and Bain (1958) reported that cell divisions continue in the epidermis until the fruit is ripe and that the cuticle is thin over recently divided cells. Thin-walled parenchyma cells underlay the epidermis of the immature fruit. The walls become somewhat thickened as the fruit matures. Within this parenchymatous tissue occur crystal idioblasts and oil glands. The first oil glands are formed in the ovary wall of the flower (fig. 1–19, *A* and *B*), and apparently these are the oil glands that are relatively small in mature fruit (fig. 1–30, *A*). Other oil glands form continually during enlargement of the fruit. The parenchyma cells adjacent to the epidermis are polyhedrons that have a tendency to become ellipsoidal as the fruit enlarges. Centripetally from the epidermis, exocarp cells become increasingly larger, and there is an increasing amount of intercellular space.

Parenchyma cells of the exocarp contain the chloroplasts (fig. 1–29, *E*), which give immature fruits their green color. During ripening, the chloroplasts are converted to chromoplasts—also called chromatophores (fig. 1–29, *A* and *F*). A breakdown of the internal membrane system and the grana-fretwork system occurs in the chloroplasts; the osmiophilic (fat) bodies increase in size and number (Thomson, 1966). The resulting loss of chlorophyll unmasks the variously colored compounds, principally carotenoids, which increase during ripening; the fruits then take on their characteristic colors (Miller, Winston, and Schomer, 1940). The carotenoids are probably in solution in the fat globules in the plastids (fig. 1–29, *F*; Dufrenoy; 1929; Thomson, 1966). The red color of blood oranges results from materials dissolved in the vacuoles (Winton and Winton, 1932–39), and the red color of Indian Red Pummelo from pink cytoplasmic crystals (Matlack, 1931).

The Mesocarp (Albedo).—The mesocarp is located in the middle of the rind and is white in most *Citrus* fruits, hence the name *albedo*. During Stage I, its cells are meristematic, polygonal in shape, and compactly arranged, and most of the increase in fruit size during Stage I is due to increase in thickness of the mesocarp with locular size increasing relatively little (fig. 1–22, *A–D*; Bain, 1958). During Stage II, cell divisions cease in the mesocarp, bulges develop on parenchyma cell walls, and schizogenous air spaces begin to form. The bulges become cylindrical arms as they continue to elongate, and thus the albedo cells become deeply lobed and the air spaces large (fig. 1–29, *B* and *C*; Bain, 1958). Distal ends of the cylindrical protuberances are joined to the distal ends of protuberances of other cells, and conspicuous pit fields occur in the walls where they join (Scott and Baker, 1947). Ramifying through the pericarp is a lattice of vascular bundles that branch from, and may later reunite with, the septal and dorsal bundles (figs. 1–22; and 1–25, *A*).

The Endocarp.—In a nontechnical sense, the endocarp is that portion of the locular membrane that sheathes the outer semispherical side of the peeled and divided fruit segment. Technically, it is the inner layer of the pericarp or the adaxial face of the hypothetical carpellary leaf. It is composed of an epidermis that lines the locules and a few adjoining layers of compactly arranged parenchyma cells (fig. 1–29, *D*). When the ovary is young, just before the petals open, the inner epidermis is a conspicuous meristematic layer (fig. 1–22, *A*), with anticlinal divisions. In Valencia orange, carpellary emergences (the "*schleimzotten*" of Tschirch and Oesterle, 1900) and juice sacs appear as the petals open. The carpellary emergences, like the juice sacs, grow into the locules; the terminal cells enlarge, the middle lamella swells, and the cells become detached (fig. 1–23, *B*; Ford, 1942). Carpellary emergences occur on septal walls and on the endocarp. Before the locules enlarge, the juice sac primordia, discussed earlier, are densely arranged. However, with expansion of the locules to full size, the stalks of the juice sacs become scattered. The endocarp cells (epidermal and subepidermal) become greatly stretched, and a thick cuticle appears on the epidermis (fig. 1–29, *D*).

The Septa and Central Axis

The septa are thin partitions between the locules (figs. 1–30, *A* and *B*; and 1–31, *A*). Each septum is composed of the two locular membranes of adjacent fruit segments with intervening spongy mesocarp-like parenchyma. In citrus fruits where segments are readily broken apart, tearing occurs in the spongy tissue. As previously mentioned, division of the fruit into regions such as the pericarp and septa is for convenience, and separate structures do not exist. Each juice-sac-filled locule is enclosed by a single locular membrane, the entirety of which the author believes should be called the endocarp. The membrane is hypothetically the adaxial surface of the infolded carpellary leaf. The locular membrane at the septum consists of a cuticle-covered epidermis and adjacent, compactly arranged parenchyma cells. In the young ovary (fig. 1–22, *A*), periclinal divisions were observed in the

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epidermis in the region of the septa, but only anticlinal divisions were seen in the endocarpal region, except of course where juice sacs were forming. The juice sacs are outgrowths from the locular membrane at its endocarpal region.

The central axis, as previously mentioned, is derived from the floral axis, and the margins of the carpellary leaf are attached to it. In the lower part of the axis, a cylinder of axillary bundles occurs; near the middle of the fruit, ovule traces diverge, and, further acropetally, the marginal carpel traces diverge. Spongy parenchyma cells with long protruding arms occur between the vascular bundles (fig. 1–25, *A*; and 1–30, *B*).

The Peduncle, Receptacle, Sepals, and Floral Disc

As the ovary transforms into the fruit and petals and stamens fall, the peduncle-receptacle region increases in diameter and becomes stemlike. The secondary vascular tissues are unlike the complex of branching and diverging whorls of primary vascular bundles characteristic of the flower and consist of a woody cylinder surrounded by secondary phloem (fig. 1–25, *A*; and 1–30, *E*). In the flower, there is an abscission zone at the base of the peduncle (fig. 1–18, *A*). In the fruit, the secondary tissues at the base of the peduncle are not adapted to abscission, because secondary xylem and secondary phloem fibers occur across the region of the former abscission zone. The constriction of the cortex found in the flower disappears. The walls of the pith parenchyma cells of the peduncle-receptacle become thickened and lignified.

Distally, the stemlike peduncle-receptacle is terminated as a dome where the whorls of carpellary bundles diverge (figs. 1–25, *A*; and 1–30, *E*). The cambium continues over the dome; xylem tissues derive from it on the proximal side; and on the distal side, phloem forms. The septal, dorsal, and axial bundles that diverge from the dome are adaptable to stretching that accompanies fruit enlargement and to abscission, because phloem and xylem fibers are absent in them and the lignified thickenings on the tracheary elements are annular or spiral. When the time comes for abscission of the fruit, the abscission layer forms just above and around the woody receptacular dome.

The sepals and the floral disc do not change greatly from the form they had in the flower (fig. 1–30, *E*).

THE SEEDLING

In a botanical sense, a plant is a seedling from the time it emerges from the seed until it forms green leaves and becomes independent of food stored in the cotyledons. In citrus, independence is considered to occur when the first foliage leaves are mature and when stem elongation commences above them.

When a citrus seed germinates, the hypocotyl and primary root are the first organs to appear (fig. 1–31, *C–D*). The hypocotyl eventually becomes about 5 mm long and remains white for some time (fig. 1–31, *G*). The primary root grows rapidly and soon becomes yellow-brown. Cotyledons of the common, commercially grown species remain underground (fig. 1–31, *G*). After the primary root is several centimeters long, the epicotyl begins to elongate by intercalary growth and reaches about 5 cm in length. The elongating shoot may lift the seed coat out of the ground if the seed was planted with the chalaza upright. While the epicotyl is elongating, the first foliage leaves expand only slightly, but thereafter they expand and mature over a period of several weeks. The foliage leaves are simple, small, ovaloid, and arranged opposite each other on the stem. The apical meristem often does not become active until the first leaves have finished expanding. The first leaf or two derived from apical growth may be simple like the first foliage leaves; the next one or two are intermediate between simple leaves and the typical unifoliate compound leaf of citrus. Leaves derived from apical growth have a spiral arrangement. In summary, growth of various parts of the citrus seedling occurs in a sequence; first, the hypocotyl-root axis grows; second, the epicotyl; third, the first foliage leaves; and finally, after about two months, stem apical growth commences, and the seedling becomes an established plant.

Hayward and Long (1942) described the anatomy of the various organs of citrus seedlings in order of their development. As stated in the section on anatomy of the root, they considered in detail the primary root, which need not again be discussed. The stem at the cotyledonary node is tender and fragile, and its vascular cylinder contains two prominent cotyledonary traces with opposite arrangement. Two smaller traces from the first leaves are also opposite each other and at 90° from the cotyledonary traces. Between these traces, in the cylinder, are provascular bundles, presumably traces of the leaf primordia in the stem tip. Below the cotyledonary node, each cotyledonary trace is broadened tangentially, and at a still lower level, each trace is divided into three collateral bundles. In the transition region, the bundles become rearranged; and, in the root below, the xylem strands occupy alternate radii to the phloem strands. After the root is several weeks old, a sharp contrast occurs in the appearance of the hypocotyl and the primary root (fig. 1–31, *F* and *G*). The hypocotyl remains shiny and white, whereas the root becomes a dull brown. The hypocotyl is covered with small, cutinized epidermal cells, among which occur stomata. On the root, stomata and cuticle are lacking, but a conspicuous hypodermis is present and, under certain conditions, also root hairs. The walls of the hypodermis and epidermis stain red with safranin, and presumably they contain suberin and wound gum (Cossman, 1939). The mesophyll of the first foliage leaves is similar to that of the later-formed compound leaves.

THE BUD UNION

In the literature of citrus anatomy, the term bud union is used in two ways. One usage refers to the fusion of tissues of a newly placed scion bud with the tissues of the axis of a small nursery tree used as a rootstock, which is usually grown from seed. The other refers to the junction on a mature orchard-tree trunk where scion tissues join rootstock tissues. The latter usage is in some respects a misnomer, since a bud is no longer involved.

Fusion of Newly Placed Buds with Seedling Stems.—Mendel (1936) made a developmental study of the fusion of buds of the Shamouti (Jaffa) orange with sweet-lime seedlings. Trees were studied in the nursery, and they were budded in the fall. The buds were inserted into T-shaped cuts on the seedling stem. The corners of bark on the stem that are pulled aside from the T-cut to insert the bud are called *bark flaps.* The bark remaining around the bud when it is cut from the bud stick is called the *shield*. In Mendel's studies, the xylem was removed from the shield before budding, so that the cambial zones of the shield and seedling were placed against each other.

Within 24 hours after budding, the ray cells on opposing surfaces of the graft mates began to swell in preparation for division. Later, other cells on the inner side of the bark and cells on the surface of the wood also began to swell and divide. Older xylem cells with lignified secondary walls did not divide. At first, the most intense callus formation occurred in the angle between the flap and the woody cylinder of the seedling; while on the shield, new cells appeared only after ten days. Callus formation progressed from under the flap toward the area under the bud. At the stage when large amounts of callus were present only under the flaps, a cavity occurred under the bud; however, after about twenty days, the cavity filled with callus tissue. When the walls of the callus cells from the graft mates met, they fused to produce one homogeneous tissue. Where tissues of the graft mates closely impinged, they fused without callus formation.

After union of the callus tissues, differentiation of the callus cells into various other kinds of cells proceeded rapidly. Within ten days, there were strands of tracheary elements and other cells with secondary lignified walls in the angle between the flaps and the xylem cylinder of the seedlings. Under the shield, lignification proceeded more slowly, with tracheids appearing at the margins of the shield after twenty days. Twenty-five or more days were required for tracheids to appear in tissues near the bud. Directly beneath the bud is a region of nonlignified cells called the basal pith plate.

Cambial reactivation or regeneration proceeded simultaneously with the differentiation of xylem from cells of the callus tissue. In places where patches of cambium had been torn from the flap or shield, regeneration of new cambial tissue occurred in the callus tissue close to the secondary bark. A cambial connection was generated in the callus between the flap and shield. Cambial regeneration under the shield was completed about one month after budding, and union of the bud with the seedling was considered accomplished.

Scion-Stock Junction.—Commercial citrus varieties are usually grown on seedling rootstock, but sometimes interstocks are also used. When graft mates are of the same species, the joint is usually difficult or impossible to locate either by external or anatomical examination, unless there happens to be some varietal difference in tissues of the mates. Navel orange, for instance, forms many more layers of phloem fibers than does the Valencia orange. When interspecific or intergeneric graft mates are used, both external and internal differences between the scion and stock tissues may be discernible. For example, the individual phloem fibers in fiber layers of sour orange tend to be coiled, like strands in a rope, whereas they are straight in Valencia orange. Furthermore, the fiber layers in sour orange are fewer in number, thicker radially, and narrower in tangential dimension than those of the Valencia (Schneider, 1954). In the lemon, phloem fiber layers are usually lacking in mature trunks, and the sieve tubes are larger than in most species used as rootstocks for lemons.

In Israel, Goldschmidt-Blumenthal (1956) studied the xylem above and below the bud union of Shamouti orange trees on nine species of rootstocks. Anatomical differences were found between scion and rootstock tissues. In the rootstocks of some of the combinations (e.g., trees on shaddock), there were fewer vessels per square millimeter of cross-section and the size of pores was smaller than in the xylem of scions of the same trees. Even the vessels in the scions were smaller than those of trees on Shamouti rootstocks.

In southern California, characteristic morphological deformities occur with certain scion-rootstock combinations which may not be detrimental to the tree. For instance, with sweet orange on sour orange rootstock, the bud union may bulge outward; when the lemon is grown on certain rootstocks, the scion may overhang the rootstock. With other combinations, the rootstock may overgrow the scion as with sweet orange on trifoliate orange *(P. trifoliata)*. Usually, there is a normal fusion of xylem and phloem in all of these cases.

All too frequently scion-rootstock combinations are grown in which the tissues are sufficiently incompatible to cause the tree to decline or die (for example, Schneider, 1956; Weathers *et al.*, 1955). Incompatibility may be induced also by virus infection; for example as with tristeza disease of sweet orange on sour orange rootstock.

GENERAL ANATOMICAL CONSIDERATIONS

Oil Glands.—Oil glands are spherical structures occurring just under the epidermis in primary tissues of the shoot (i.e., leaf, thorns, prophylls, sepals, etc.). When pressure is applied to tissues containing glands, a liquid containing essential oils squirts or oozes out. The first oil glands differentiate very early in newly forming organs and others are initiated while the organ is maturing. Evidence of oil-gland differentiation is first apparent when the cytoplasm of a cluster of cells becomes much thickened and stains darkly. Then cell divisions occur in which the cell plates are oriented perpendicular to radii from the center of the gland. The inner cells of the glands enlarge greatly into turgid, polyhedral, thin-walled parenchyma cells. The cells surrounding the large polyhedral central cells become lunar-shaped, as seen in median sections of the glands (Fohn, 1935). Often in sections, the internal polyhedral cells are broken down and only a cavity remains. It is not known whether this results from (1) a natural lysigenous process, (2) breakage due to pressure applied to the glands, or (3) as an artifact of fixing and sectioning. In some sections made by the author, many of the polyhedral cells were intact (fig. 1–30, *A*). Dede (1958) refers to the oil glands of the Rutaceae as secretory cavities; as noted above, the structures are more complex than mere cavities. Since the term oil gland is well established in citrus literature, it is used in this chapter.

Cellular Contents.—Some kinds of citrus cells do not have a living protoplast at maturity. This is true of sclerids, fibers, and some parenchyma cells. Semi-living cells are the sieve tubes and tracheary elements. The sieve tubes are enucleate, but contain cytoplasm and have protoplasmic connection to the companion cells. Although the tracheary elements are without cytoplasm, they are enclosed by living parenchyma cells that apparently have protoplasmic protrusions extending through the pit pairs to the tracheary element.

Cytoplasmic organelles include the nucleus, the mitochondria, and various plastids. Meristematic cells have a very thick cytoplasmic layer, whereas the cytoplasm of differentiated cells is variable in thickness. For instance, the outer thin-walled cells in the cortex of the stem have conspicuous cytoplasm that contains chloroplasts, whereas the large, thick-walled inner cells have a very thin layer of cytoplasm. Characteristics of the cytoplasm have been described elsewhere throughout this chapter.

The vacuole has been described as a cavity in the cytoplasm containing a watery liquid called the cell sap (Jackson, 1927). Following fixation with chromic-acid-containing fixing solutions, the vacuoles of some cells contain globular bodies. These were considered to be artifacts by Schneider (1957). Another type of globular bodies called coacervates occur in vacuoles of parenchyma cells of orange trees affected by mottle-leaf; these are not artifacts (Dufrenoy and Reed, 1942). Dufrenoy and Reed report: "The coacervate is a body rich in colloids and immersed in a liquid relatively poorer in colloids, which has been designated the equilibrium liquid." Surrounding the coacervates is a precipitation membrane made up of phosphotids, a complex of lipoids and phosphorus. The coacervates described by Dufrenoy and Reed were usually only one or two in a cell. Vacuolar bodies, apparently of the two types just described, have been recently interpreted as small and large spores of *Physoderma citri* sp. nov.; several citrus species are said to be regularly infected (Childs, Kopp, and Johnson, 1965).

Distinctive Anatomical Features of Some Species.—Swingle (1909) reported that *Poncirus trifoliata* rootstocks of nursery trees can be distinguished from sour orange rootstocks by the presence of cells with thickened walls in the pith. Carpenter (1961) reported that there are root primordia in the stem of the citron (*C. medica* L.) and in hybrids of citron with other species.

ACKNOWLEDGMENTS

This chapter is for the most part original, but certain excerpts in revised or identical form have been taken from Chapter III, "General Morphology, Histology, and Physiology," by E. T. Bartholomew and H. S. Reed in Volume I of the 1943 edition of *The Citrus Industry*. Peter Sasaki prepared many of the sections and aided in macrophotography. Kenneth Middleham made the photographs for A, B, and C of figure 1-1. Sections in figures 1-11 and 1-12 were prepared by Mrs. Johanna Barr. Mr. Charles S. Papp made the three-dimensional drawings from the author's sketches and prepared the overlays for the figures.

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Fig. 1–1. *A–D*, spring-cycle shoots of sweet orange (*Citrus sinensis* [L.] Osbeck). *A*, sterile spring-cycle growth. Phyllotaxy 3/8, with spirality to the right. Spring-cycle leaves are numbered consecutively, with leaf 8 occurring directly above one selected arbitrarily as *n*. (See fig. 1–3, *A*.) *B*, spring-cycle shoot with leaves and flower buds. First leaf below terminal flower is aborted. Axillary flowers are smaller than the terminal one. Spirality to the left. *C*, in the axils of leaves of a previous growth flush are spring-cycle flowering branches (sometimes called inflorescences), each with leaves or aborted leaves having spirality to the right. They are numbered 3–6 and occur in the axils of leaves of a shoot of a previous flush whose leaves are correspondingly numbered. Spirality of leaves on older flush is to left (see arrows). Shoot 5 has four flowers in the axils of well-developed leaves, and in shoot 4 there is but one. Other flower buds are in the axils of aborted leaves, some of which have abscissed. *D*, dead apex of spring-cycle growth that will abscise. Remaining stub is thornlike. *E*, stem apex of lemon (*Citrus limon* [L.] Burm. f.) which was cleared and then stained with safranin to illustrate the continuity of vascular bundles from leaf to vascular cylinder of stem. Details are: *L*, one of the lateral leaf traces; *m*, median leaf trace; *a*, abscission zone. (*A*, ×1.1; *B*, ×1.7; *C* ×0.8; *D* ×5; *E*, ×20.)

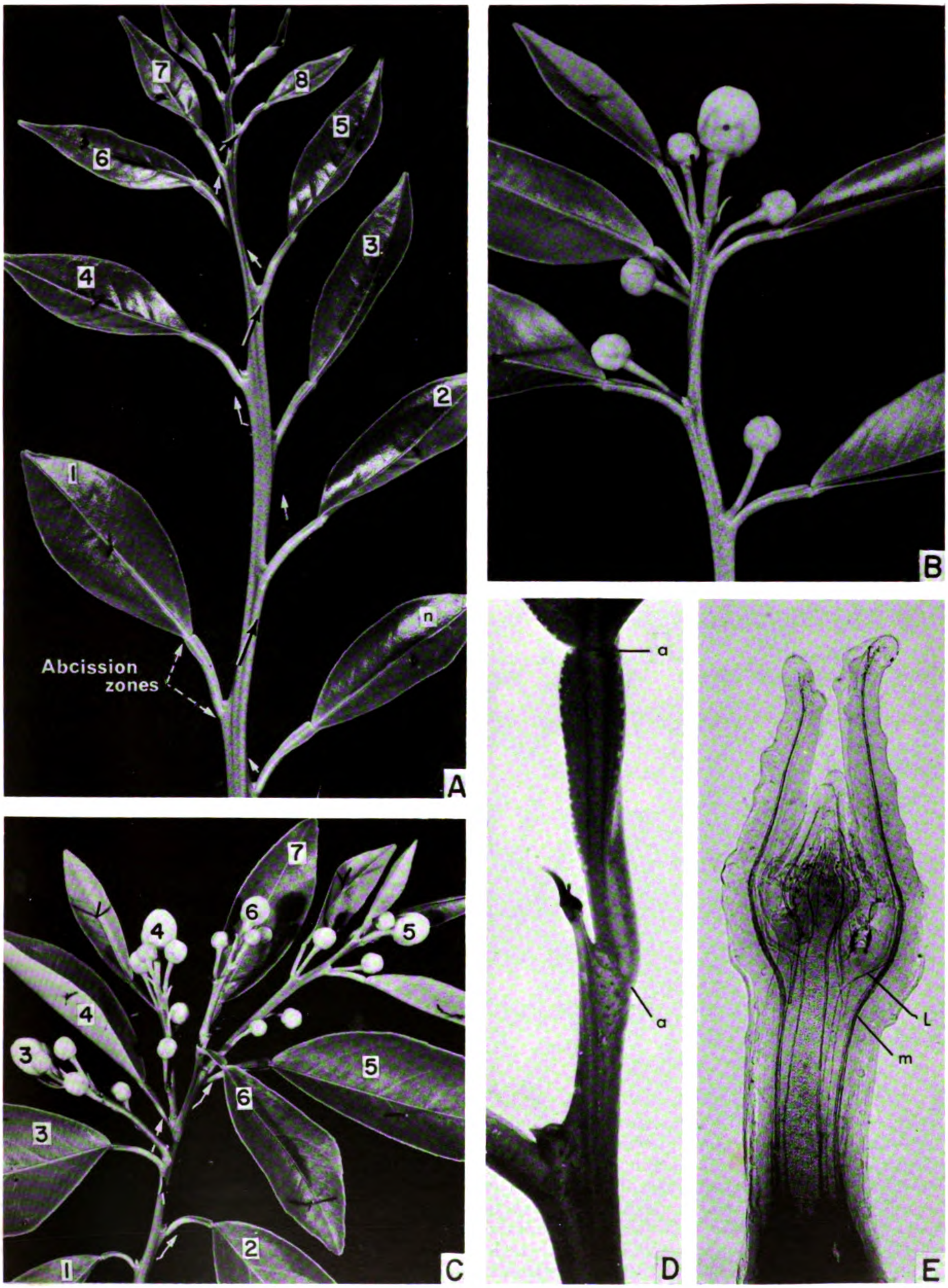
7
8
5
6
3
4
2
1
n
Abcission zones
A
B
7
4
6
5
4
3
5
6
3
2
1
C
a
a
D
L
m
E

Fig. 1–2. Sections of lemon stem. *A*, longitudinal section of actively growing stem tip. The younger leaf primordia (3, 4, 6) are curved over the apical meristem. A procambial strand (with tracheary elements which are not visible at this magnification) has differentiated in primordium 3. *B*, cross-section of axillary bud of older shoot. An accessory axillary bud has formed in the axil of prophyll 7 and two of its prophylls (*a* and *b*) are shown. *C* and *D*, median leaf traces of primordium number 5 of figure 1–3. *C*, trace 5 at the level of figure 1–3,*B*. There is an immature sieve-tube element and two immature tracheary elements. *D*, trace 5, 430 microns down the stem from *C* at the level of figure 1–3,*C*. There is a mature sieve tube, a mature tracheid, and an immature tracheary element. (*A*, × 100; *B*, × 60; *C*, × 1,700; *D*, × 1,100.)

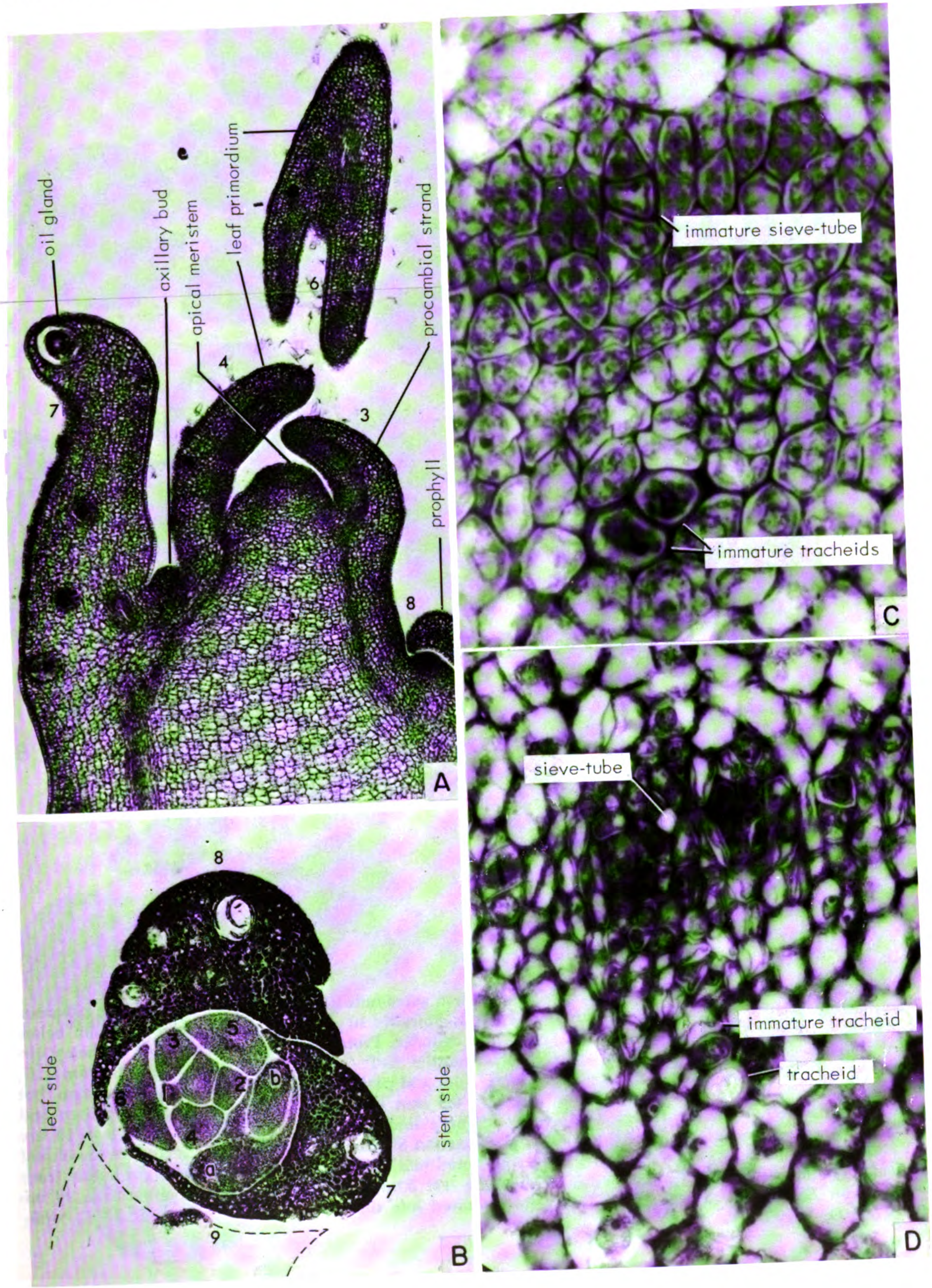
oil gland
axillary bud
apical meristem
leaf primordium
procambial strand
prophyll
6
4
7
3
8
A
8
leaf side
stem side
3
5
2
b
6
4
a
7
9
B
immature sieve-tube
immature tracheids
C
sieve-tube
immature tracheid
tracheid
D

Fig. 1–3. Lemon shoot. *A*, tip of the apical meristem surrounded by successively older leaf primordia. The most recently formed primordium is numbered 1. Spirality to right. *B*, cross-section 0.25 mm below *A*. Traces of primordia 1–7 organized into a vascular cylinder. Protophloem sieve tubes and tracheary elements have been initiated in the median trace of primordia 5–7 (not visible at this magnification). *C*, cross-section 0.4 mm below *B*. Tissues of the axillary bud and petiole of leaf 13 are joined to the stem, but their vascular cylinders are diverged. *D*, cross-section 0.1 mm below *C*. Traces from leaf 13's thorn and axillary bud have become incorporated into the vascular cylinder and the lateral bundles of leaf 13 are partially diverged from the median bundle. *E*, cross-section 80 microns below *D*. The median trace of leaf 13 and its lateral traces (*L*13) are partially incorporated into the vascular cylinder. The right lateral trace of leaf 13 enters the vascular cylinder just beyond the leaf 5 median trace (*M*5). *F*, cross-section 90 microns below *E*. Vascular traces of leaf 13 are incorporated into the vascular cylinder. (*A*, *B*, *E*, *F*, ×80; *C*, ×60; *D*, ×105.)

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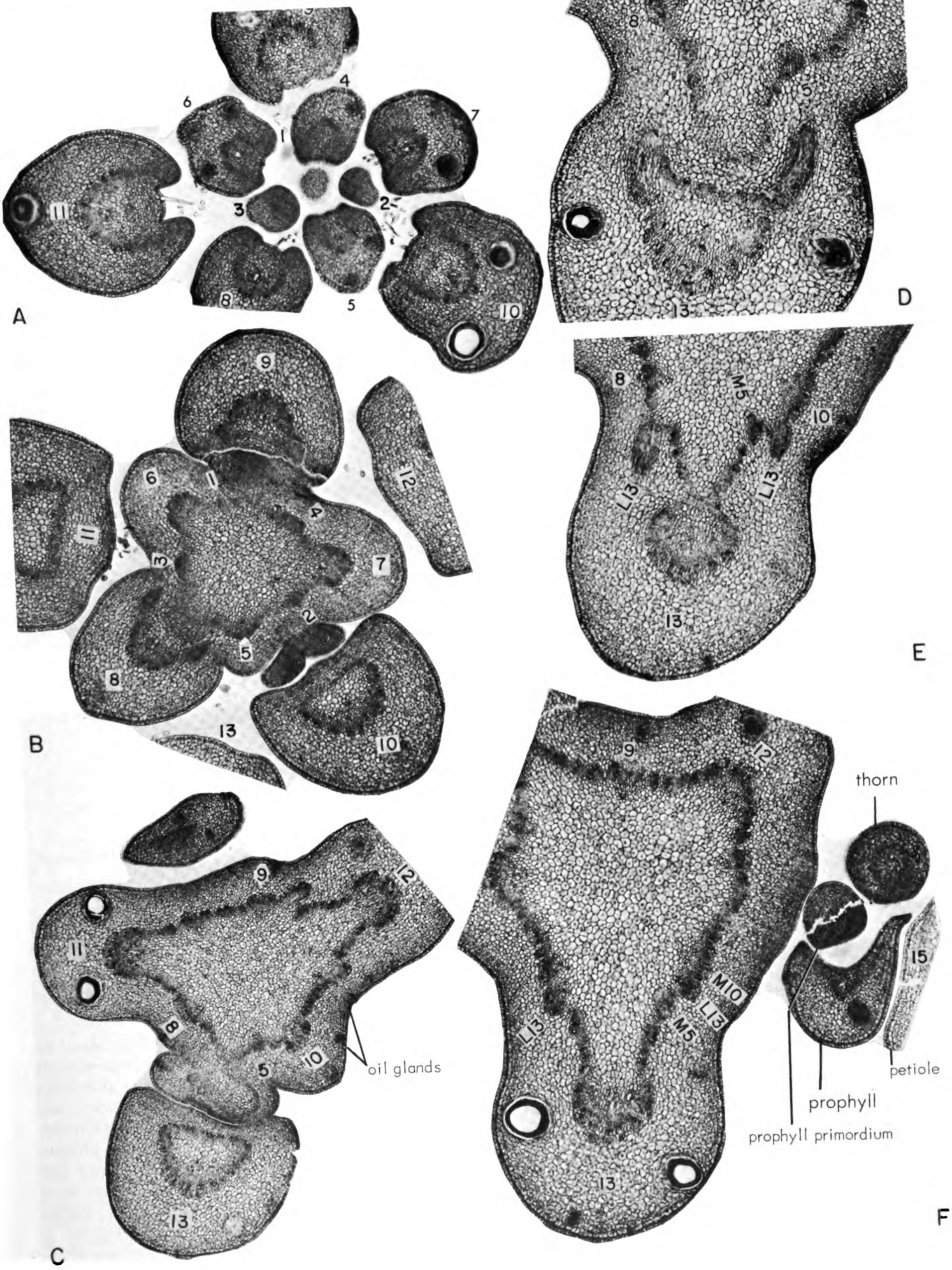
A
1
2
3
4
5
6
7
8
9
10
11
B
1
2
3
4
5
6
7
8
9
10
11
12
13
C
5
8
9
10
11
12
13
oil glands
D
5
8
13
E
8
M5
10
L13
L13
13
F
9
12
M10
L13
M5
L13
13
thorn
15
petiole
prophyll
prophyll primordium

Fig. 1–4. Cross-sections from various levels of a green, actively growing Ledig Lisbon lemon stem 60 cm long. *A*, approximately 0.4 mm from the apical meristem. The tracheary elements shown are a part of the 8th leaf trace. *B*, from the 21st internode. The cortex is mature. There are an epidermis, inner and outer layers of cortex with oil glands, a ring of immature primary phloem fibers, metaphloem, procambium, primary xylem, and pith. *C*, from the 37th internode. Some of cortical cells of the inner layer have collapsed. Primary phloem fibers are mature, the metaphloem is crushed against them, and a ring of secondary phloem has been produced. *D*, the stem has further increased in diameter, the ring of primary phloem fibers has been fragmented by tangential stretching of the bark, and divisions of the interfascicular parenchyma have occurred. Meristematic activity concerned with dilatation is becoming localized (see arrow). (After Schneider, *Amer. Jour. Bot. 42*:894.)

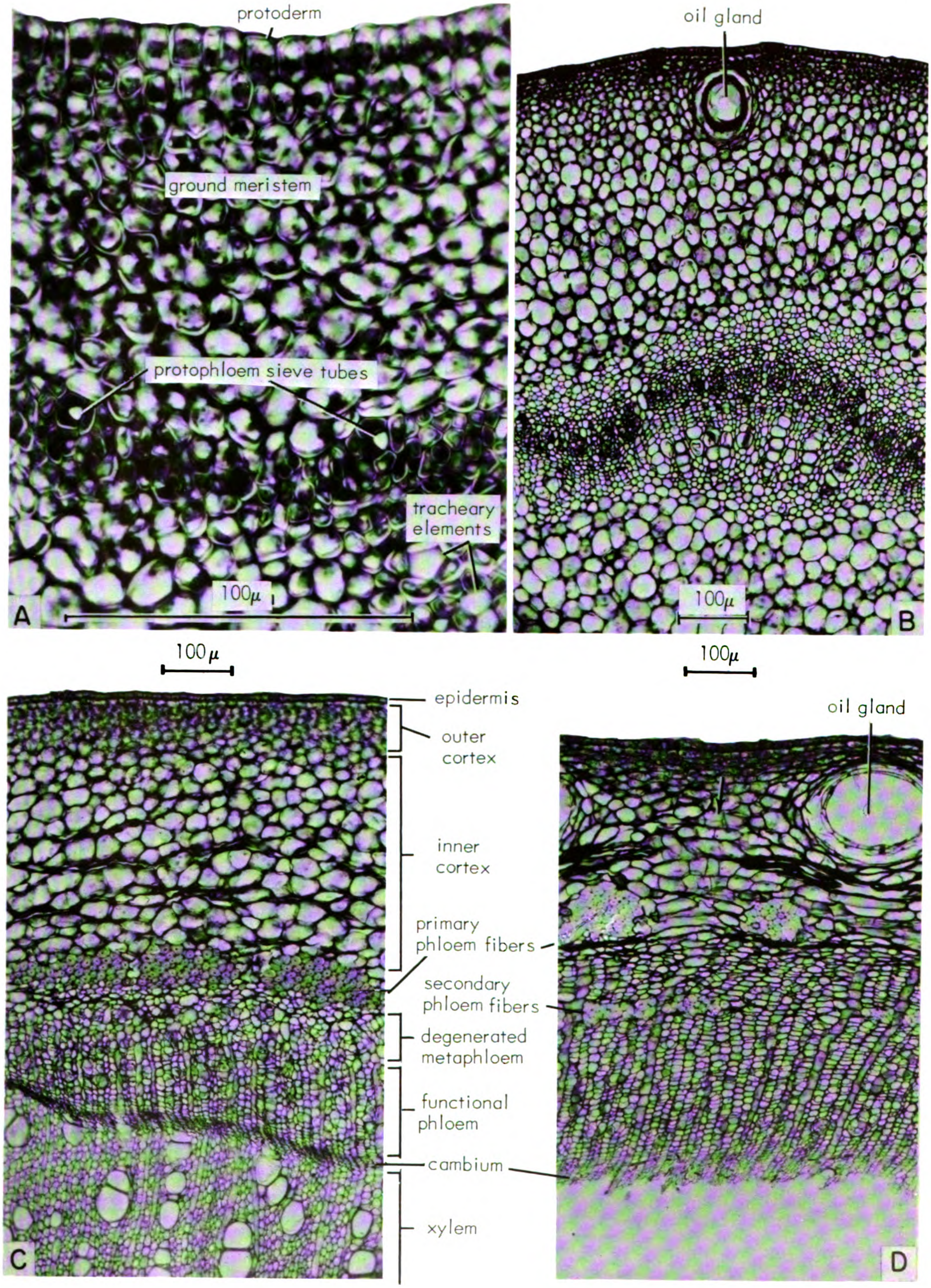
protoderm
ground meristem
protophloem sieve tubes
tracheary elements
100μ
A
oil gland
100μ
B
100μ
100μ
epidermis
outer cortex
inner cortex
primary phloem fibers
secondary phloem fibers
degenerated metaphloem
functional phloem
cambium
xylem
C
oil gland
D

Fig. 1–5. Sections of lemon bark. *A*, from a stem, 1 cm in diameter. Dilatation tissue (between the broken lines) containing sclereids (*s*) has a periderm over it. *B*, bark from the underside of a scaffold branch. The nonfunctioning phloem has become fragmented by dilatation tissue, some recently produced (+ signs). The rectangle outlined by the broken line is magnified in figure 1–10, *C*. *C*, cross-section of epidermis with immature guard cells. *D*, cross-section of fully developed stoma. Lightly stained cuticle overhangs the stomatal opening (arrows); between cells it intrudes and appears as a wedge (*w*). Epidermal cells have a darkly stained, thickened outer-tangential wall (*t*). Guard cells are at *g* and subsidiary cells at *s*. *E*, tangential view of darkly stained cuticular wedges (*w*), epidermal cells (*e*), subsidiary cells (*s*), guard cells (*g*), and stoma plug (*p*). *F*, cross-section showing cuticle, epidermis, and outer cortex. *G*, surface view of cuticle of lemon stem stained with safranin and photographed with light of a wavelength (510–540 mμ) absorbed by safranin. The outer tangential walls of the oil-gland cover cells were free of safranin, and the cuticle which intruded between cells was pitted. (After Schneider, *Amer. Jour. Bot.* 42:896, 898, 904.)

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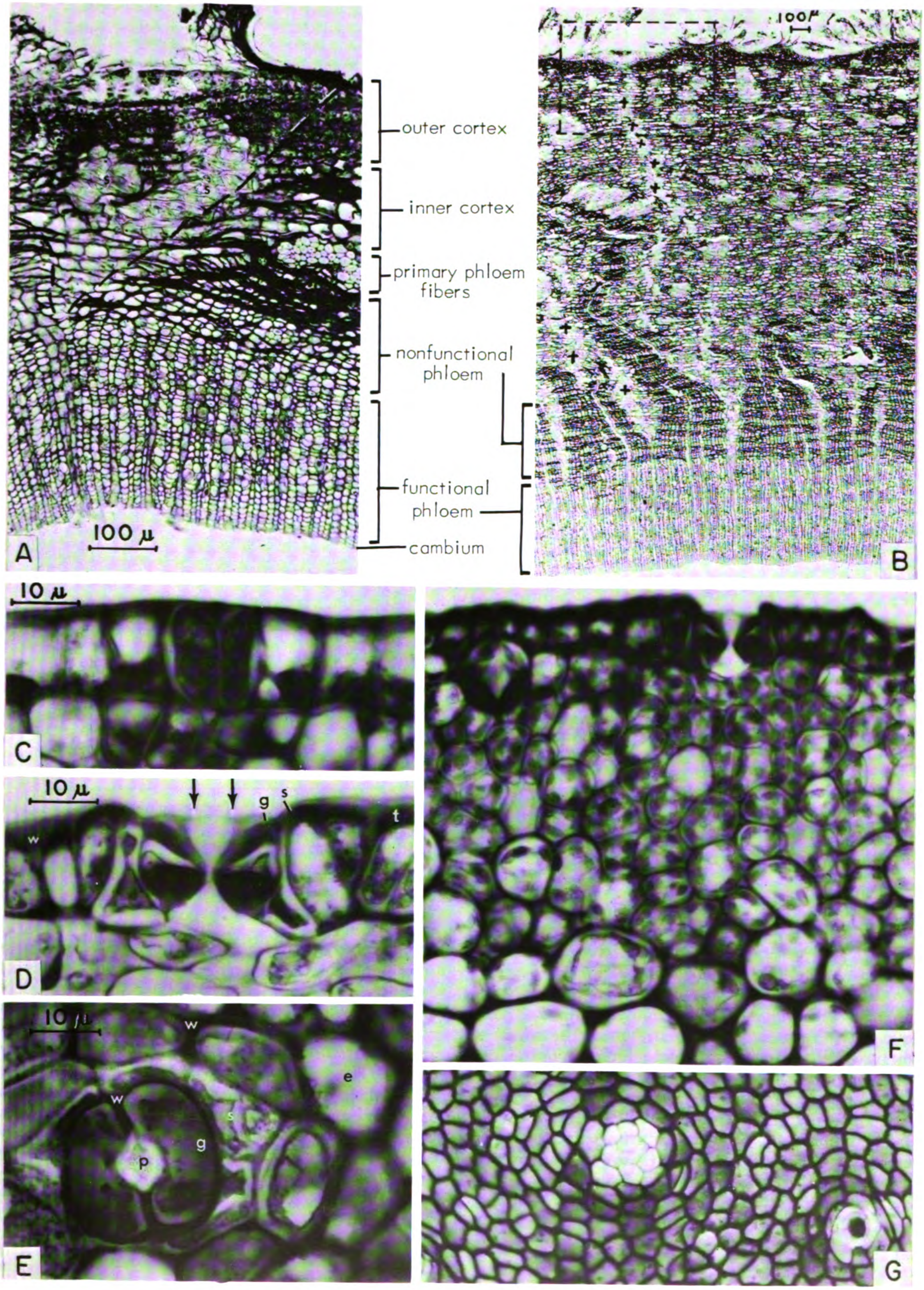
s
s
outer cortex
inner cortex
primary phloem fibers
nonfunctional phloem
functional phloem
cambium
100 μ
A
100μ
B
10 μ
C
10 μ
w
g
s
t
D
10 μ
w
w
s
e
g
p
E
F
G

Fig. 1–6. Sections of primary tissues of lemon stem. *A*, longitudinal section of protoxylem (leaf trace 8 of figure 1–2, *A* at 4.5 mm from stem tip). Section was photographed with polarized light; wall thickenings are white. Annular thickenings (*a*) of tracheary element stretched by stem elongation have become widely separated; the element is partially collapsed. Tracheary elements with coarse, out-of-focus, helical thickenings are mature and slightly stretched; the adjacent elements with fine, closely-placed thickenings are immature and still contain nuclei which are out of the plane of focus. *B* and *C*, sections of internode number 30 at approximately 8 cm below the apex. Sections were from the same stem as those illustrated in figure 1–3. Metaphloem and metaxylem are nearing the height of their development; secondary phloem was present, eight internodes below. *C*, end walls of immature vessel members are swollen prior to perforation. *D*, median trace of the 8th leaf primordium at 0.5 mm below the stem tip. There are protoxylem tracheary elements (*t*) and protophloem sieve tubes. Procambium occurs between the xylem and phloem. From the arrangement of cells around the protophloem sieve tube (indicated by dashes), it appears that they were derived by periclinal division from the procambial cells, which also gave rise to the sieve tubes. (*A*, ×1,100; *B*, ×200; *C*, ×250; *D*, ×1,200.)

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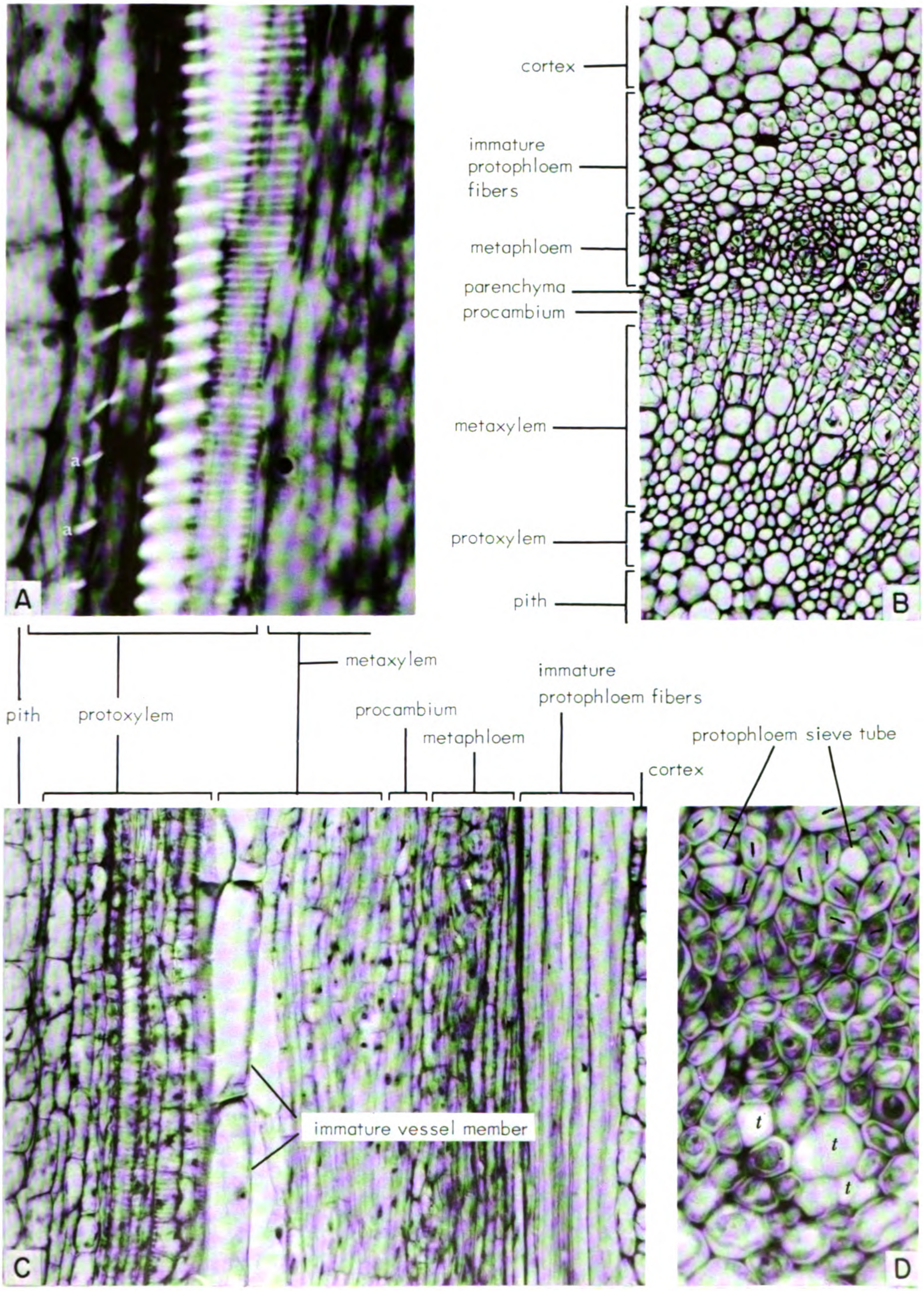

A
a
a
cortex
immature protophloem fibers
metaphloem
parenchyma
procambium
metaxylem
protoxylem
pith
B
pith
protoxylem
metaxylem
procambium
metaphloem
immature protophloem fibers
cortex
protophloem sieve tube
immature vessel member
C
t
t
t
D

Fig. 1–7. Sections of sweet-orange-trunk bark collected in April, 1947. *A* and *B*, transverse and radial sections, respectively, showing a small portion of xylem, the cambial zone, the functional phloem, and a small portion of nonfunctional phloem. *C* and *D*, tangential sections of the cambial zone and functional phloem, respectively. (*A*,*B*, ×120; *C*,*D*, ×300.)

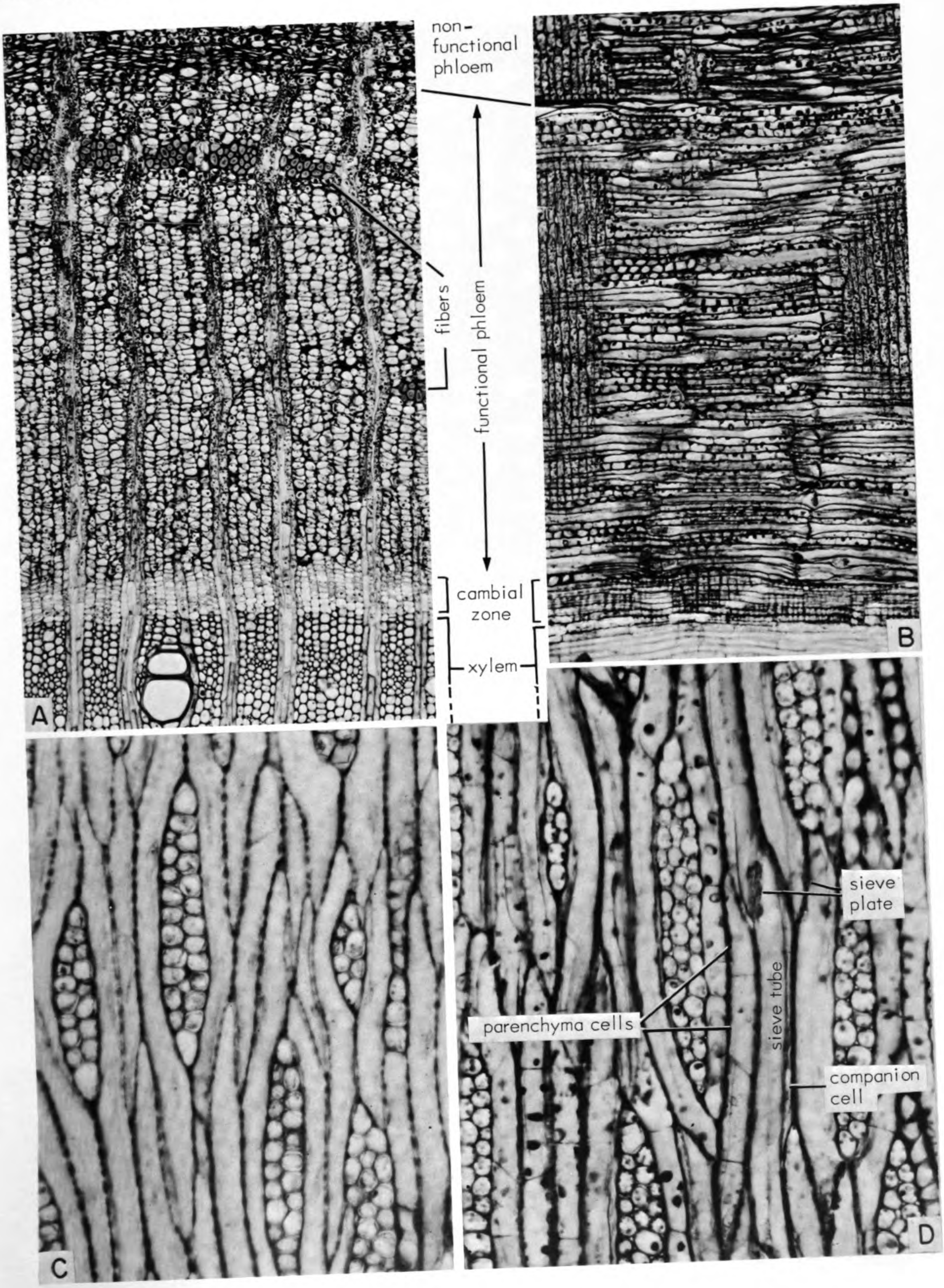

non-
functional
phloem
fibers
functional phloem
cambial
zone
xylem
A
B
C
sieve
plate
parenchyma cells
sieve tube
companion
cell
D

Fig. 1–8. Xylem of sweet orange. *A*, cross-section of the tree trunk collected Oct. 10 showing possibly five annual rings. Each annual ring is terminated by a row of terminal parenchyma cells. Cambium on the left. The first wood produced each year (i.e., to the left of each row of terminal parenchyma cells) lacks the metatracheal parenchyma (black and white arrows) found in later wood. Vessels are only occasionally found in the terminal parenchyma; they are usually small (white arrows), whereas those found in metatracheal parenchyma are often large. *B* and *C*, macerated secondary xylem of sweet orange twig. (*A*, ×24; *B*, ×120; *C*, ×250.)

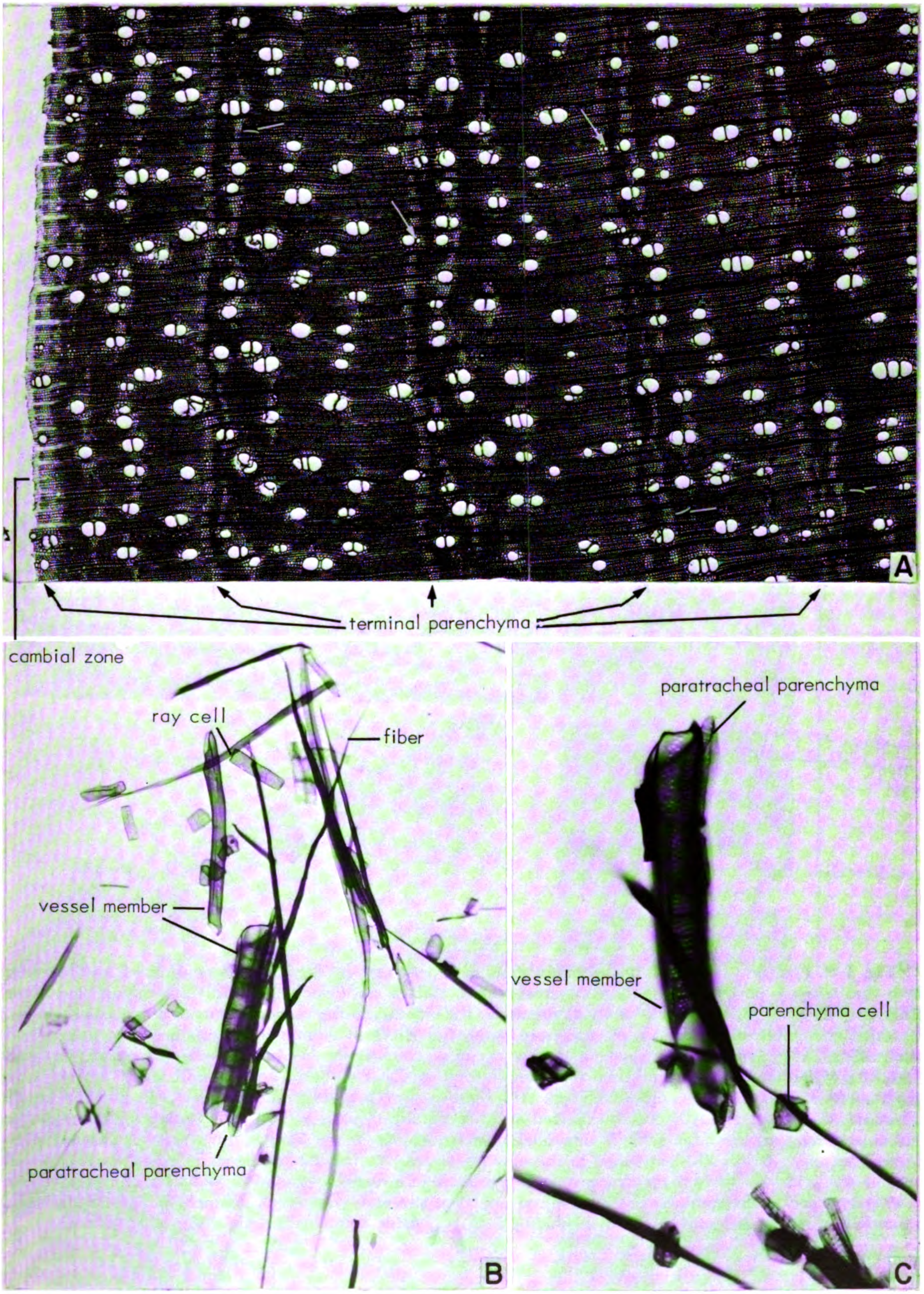

A
terminal parenchyma
cambial zone
ray cell
fiber
vessel member
paratracheal parenchyma
B
paratracheal parenchyma
vessel member
parenchyma cell
C

Fig. 1–9. Periderm formation in the lemon stem. *A* and *B*, vertical cracks in the cuticle of an enlarging stem that triggers periderm formation. *A*, cross-section stained with Sudan IV. Only the cuticle and outer tangential wall of epidermal cells stained. Breaks in the cuticle are the vertical, elongated expansion cracks. *B*, cuticular remains after removal of cells from tangential slices of stem with a mixture of chromic and nitric acids; cuticle stained with safranin. At lower end of crack there was hypertrophy of parenchyma and stretching of cuticle; at upper end there was simple tearing. *C*, at *a* there occurs meristematic activity and hypertrophy of cells under an epidermal crack. At *b* there are two hypertrophic epidermal cells and the cuticle is broken. *D*, phellogen has become organized and has produced orderly arranged phellem cells under the earlier produced hypertrophic cells. *E*, new phellogen is forming in the phelloderm of an established periderm. The phelloderm cells centrifugal to the new phellogen are forming secondary lignified walls and will become phelloids. *F*, lower magnification of the periderm in a recently expanded portion of the stem. There is a layer of cork cells adjoining the phelloderm and then successive layers of parenchyma, phelloids, cork, parenchyma, etc. *G*, tangential section of phellogen cells. (After Schneider, *Amer. Jour. Bot.* 42:902.)

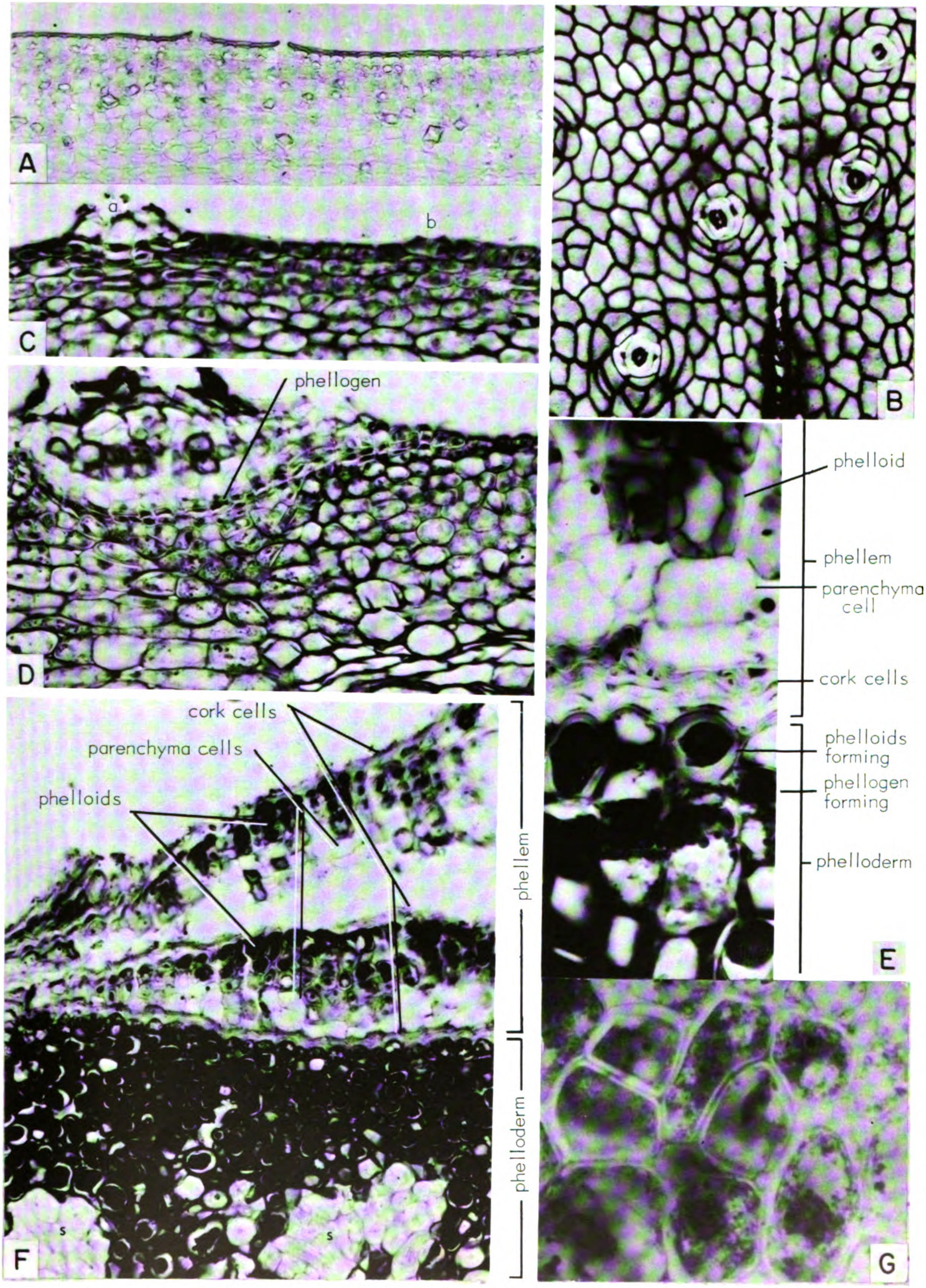

A
B
a
b
C
phellogen
D
phelloid
phellem
parenchyma cell
cork cells
phelloids forming
phellogen forming
phelloderm
E
cork cells
parenchyma cells
phelloids
phellem
phelloderm
s
s
F
G

Fig. 1–10. Lemon bark. *A*, cross-section of a newly activated dilatation meristem (*d*) in dilatation tissue. *B*, tangential section of older dilatation meristem (*d*) in dilatation tissue. *C*, cross-section of recently formed dilatation tissue (*d*) terminating at an active phellogen. At the left and the right edges of the photograph there is no phellogen activity. *D*, external view of trunk phellem. Light-colored areas are recently formed phellems. Details: (*d*) dilatation meristem, (*s*) sclereids, (*p*) degenerated dilatation parenchyma. (*D*, ×4.5.) (From Schneider, *Amer. Jour. Bot.* *42*:904.)

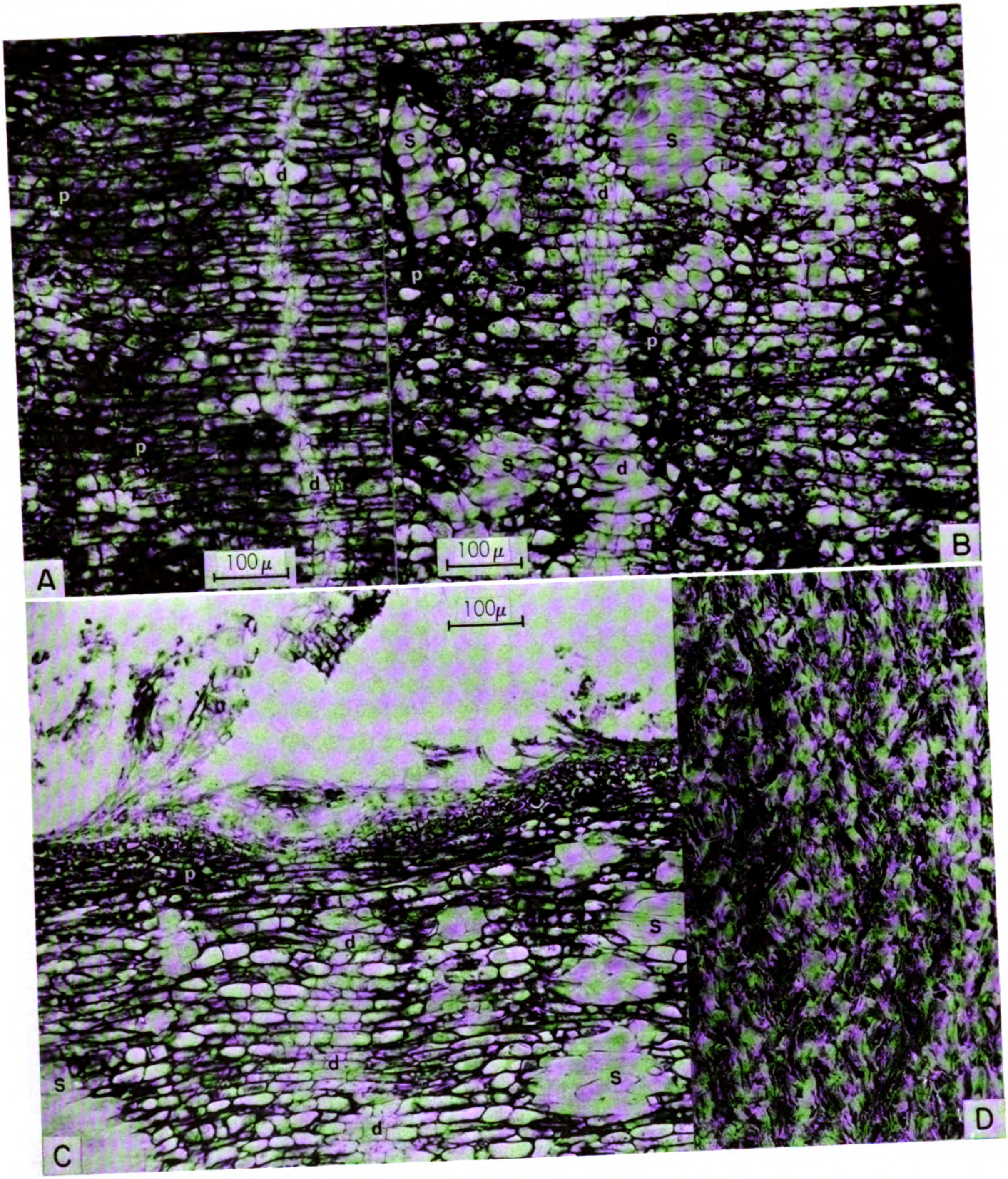

p
d
p
d
100μ
A
s
s
d
p
p
s
d
100μ
B
100μ
p
p
d
s
d
s
s
d
C
D

Fig. 1–11. Sweet orange leaves. *A*, adaxial side of a portion of an immature leaf which was cleared, stained with tannic acid and safranin, and mounted in balsam. Midvein at left. Major lateral veins indicated by arrows. *B*, paradermal section through spongy mesophyll and veins showing mesophyll islands, vein endings, and oil glands. *C*, higher magnification of a part of *B*. Bundle-sheath cells surround the veinlets. Area in rectangle is shown in *D*. *D*, higher magnification of *C*. Sieve tube and tracheary element are shown among elongated parenchyma cells. (*A*, ×16; *B*, ×100; *C*, ×460; *D*, ×1,150.)

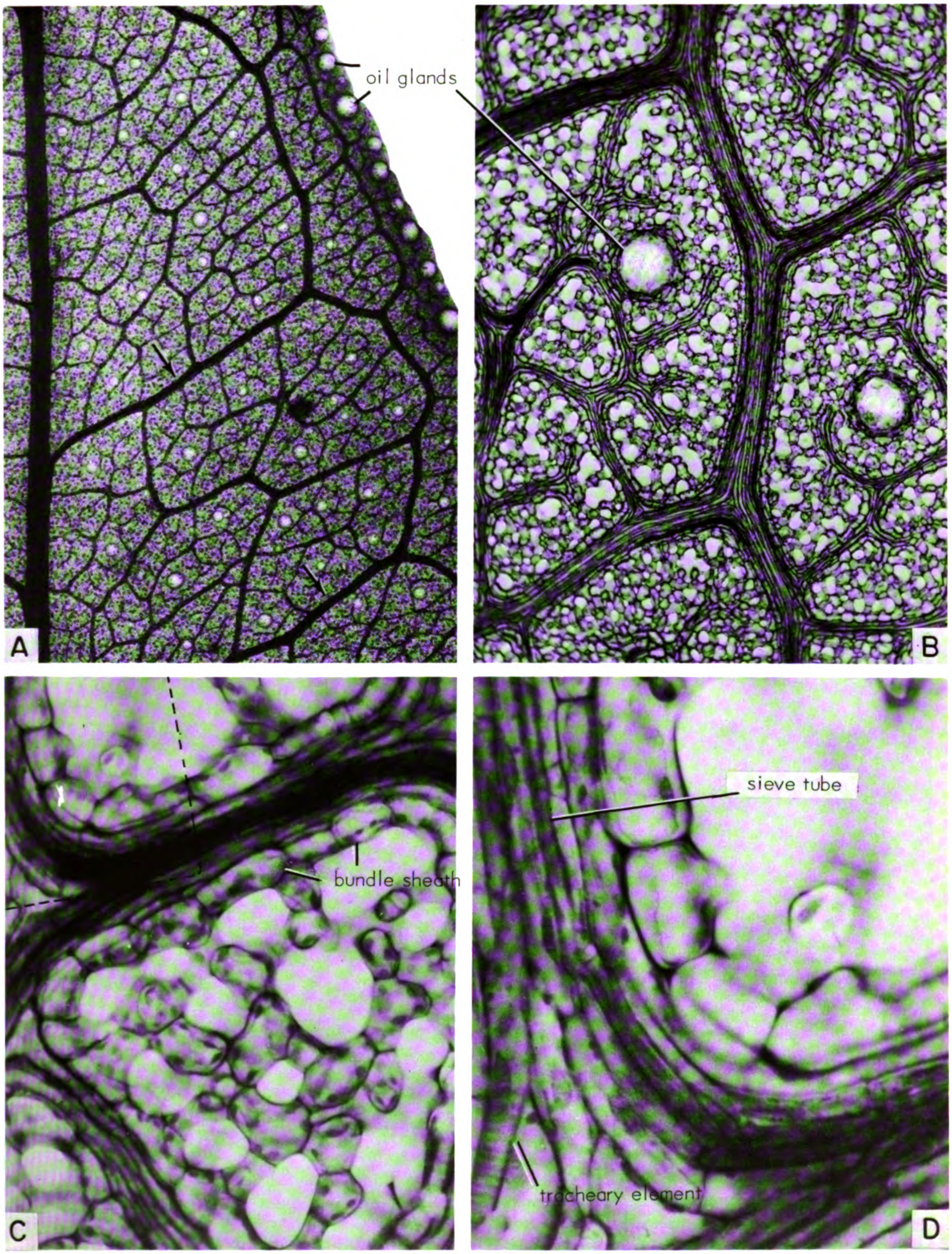

oil glands
bundle sheath
sieve tube
tracheary element
A
B
C
D

Fig. 1–12. *A*, cross-section of a major lateral vein of the leaf of greenhouse-grown, sweet orange seedling. *B*, oil gland and tiny veinlets in cross-section of field-grown lemon leaf. *C*, cross-section of lower surface of sweet orange leaf showing stoma and substomatal chamber. *D*, paradermal section of upper surface of leaf. *E*, paradermal section just above lower epidermis showing armed type of cells and intervening air spaces which occur above stomata (arrows). *F*, lower epidermis. (*A*, ×115; *B*, ×320; *C–F*, ×1,100.)

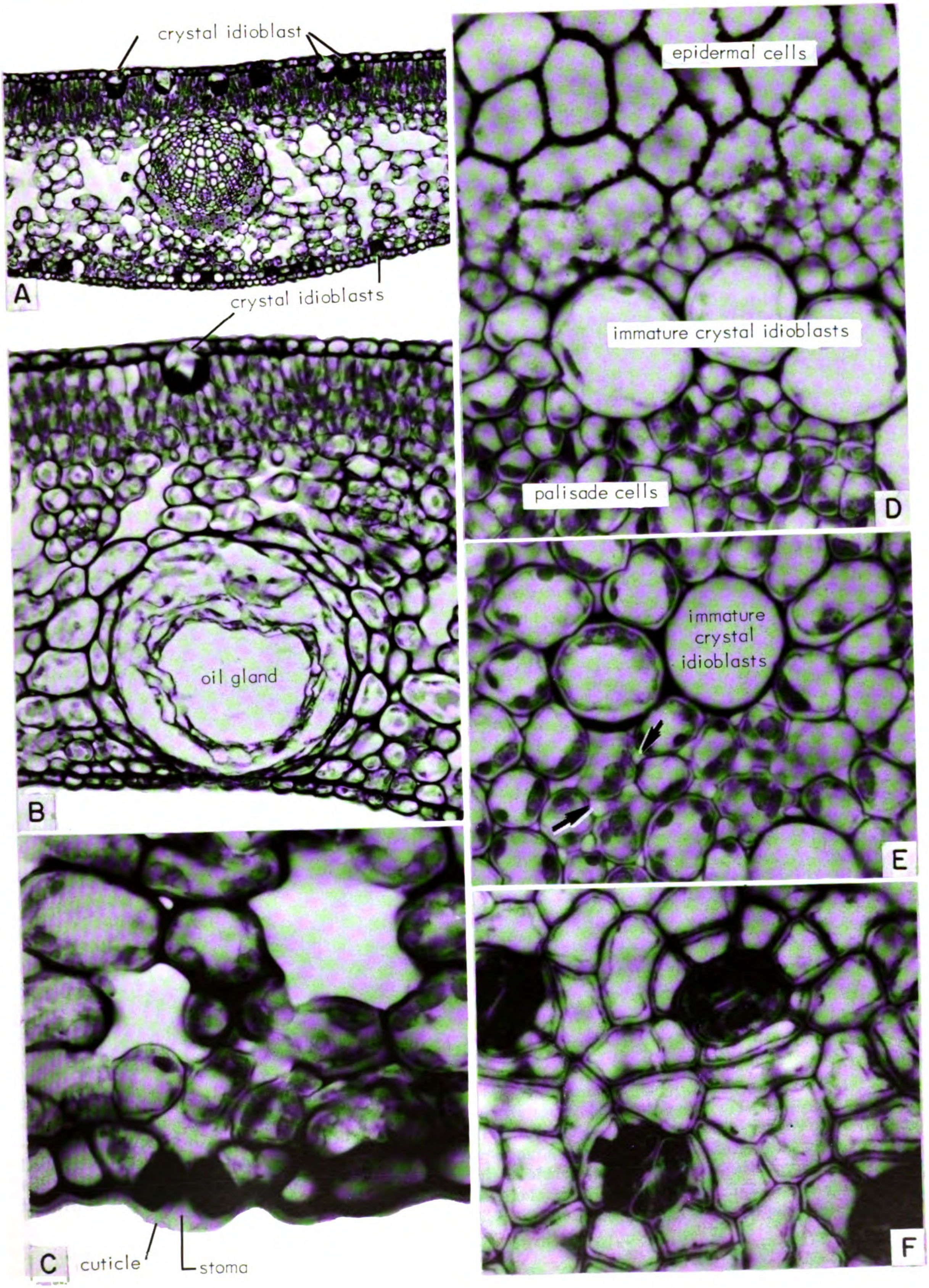

crystal idioblast
A
crystal idioblasts
B
oil gland
C
cuticle
stoma
epidermal cells
immature crystal idioblasts
palisade cells
D
immature crystal idioblasts
E
F

Fig. 1–13. Leaf sections. *A*, longitudinal section of the lamina-to-petiole abscission zone of sweet orange seedling. Lignified walls intensely black. Note absence of fibers and elongated parenchyma cells in the abscission zone. Vessels follow a zig-zag course through the abscission zone and some appear in cross- or oblique-section (arrow). *B*, cross-section through lamina-to-petiole abscission zone of lemon leaf. Section shattered upon sectioning, whereas sections to either side of the abscission zone did not. *C*, oil-gland cover cells in the upper epidermis of Eureka lemon leaf. Cuticular wedges (*w*) between the epidermal cells that surround the cover cells photograph intensely black. (*A*,*B*, ×32; *C*, ×1,100.)

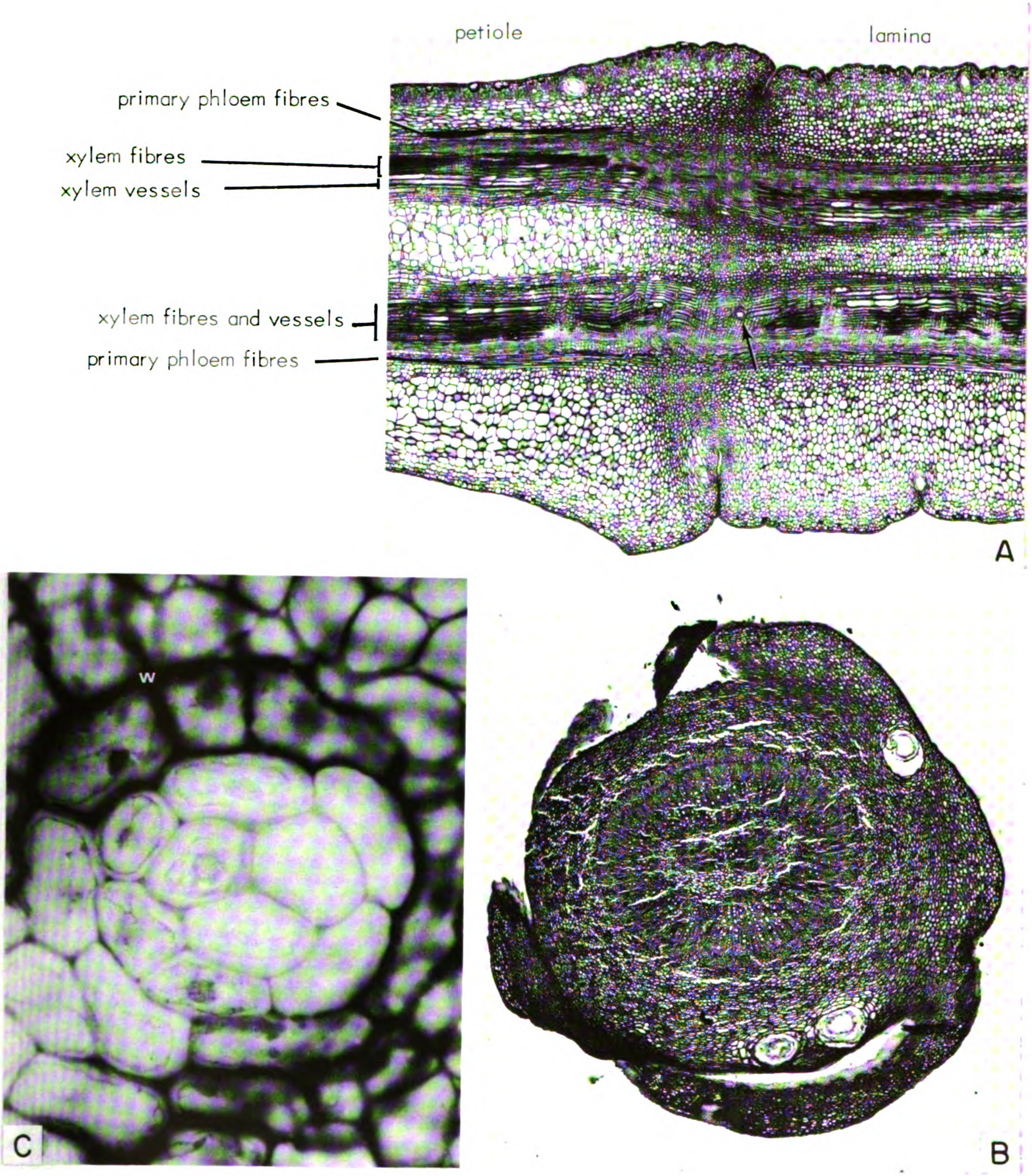
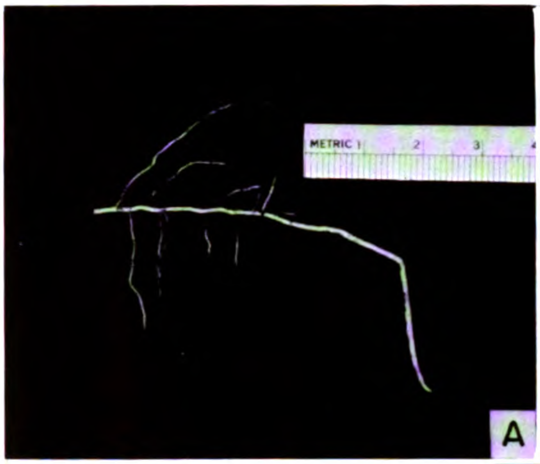
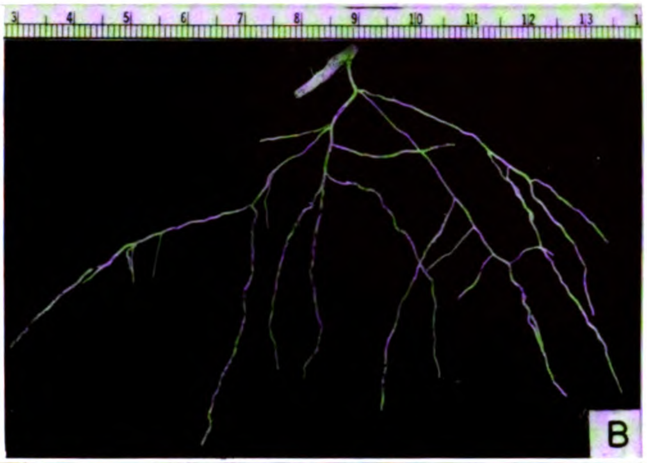
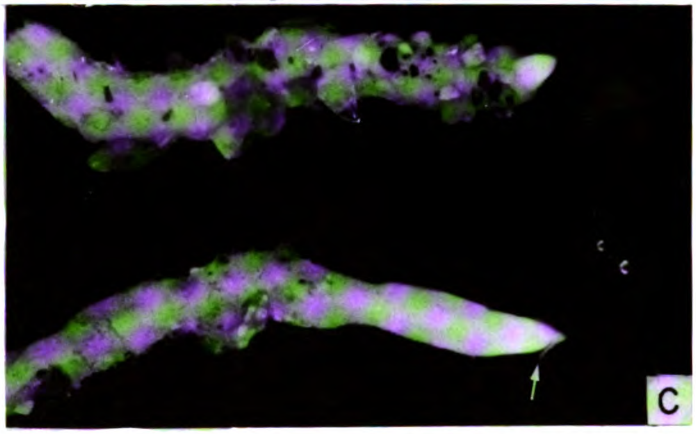
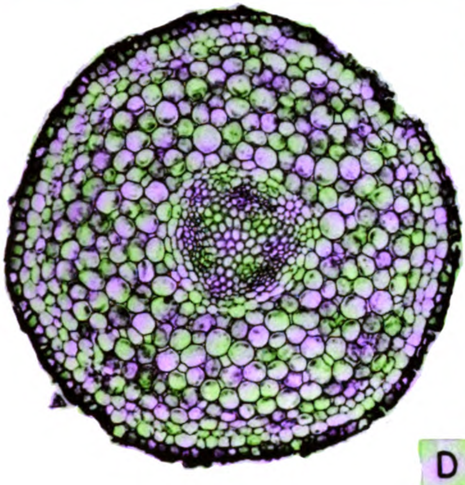

petiole
lamina
primary phloem fibres
xylem fibres
xylem vessels
xylem fibres and vessels
primary phloem fibres
A
w
C
B

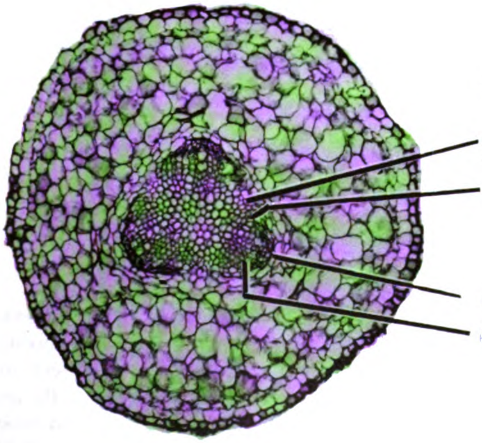
Fig. 1–14. *A*, newly elongated pioneer root of *Citrus depressa* Hay. with newly forming fibrous roots. *B*, bunch of fibrous roots of sweet orange attached to a pioneer root. *C*, sweet orange root tips. The upper one is resuming growth; the lower one is constricted where growth was earlier resumed. The rootcap is cone-shaped (arrow). *D–F*, cross-sections of *Citrus depressa* root. *D*, mature triarch fibrous root with no secondary growth. Metaxylem is at the center of root. Primary phloem strands alternate with the protoxylem ridges. *E*, triarch fibrous root in which secondary xylem and phloem have formed centripetal to the primary phloem strands and caused the nonfunctional primary phloem to be flattened. *F*, pentarch pioneer root. Note that the cortex has more layers of cells than the triarch fibrous roots and that a large pithlike area has differentiated from the plerome. (*C*, ×16; *D–F*, ×120.)


METRIC 1 2 3 4
A


3 4 5 6 7 8 9 10 11 12 13
B


C


D


E

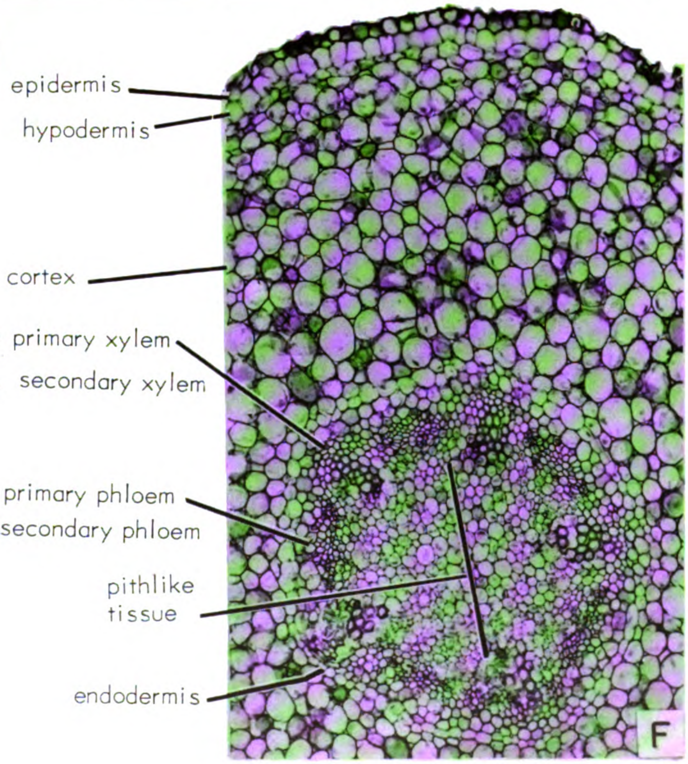

epidermis
hypodermis
cortex
primary xylem
secondary xylem
primary phloem
secondary phloem
pithlike tissue
endodermis
F

Fig. 1–15. *A* and *B*, two magnifications of a median section of root tip of Mexican lime (*Citrus aurantifolia* [Christm.] Swing.) which is ceasing to grow. The large periblem cells between the tip of the plerome and the layer of cells designated dermatogen-calyptrogen divide periclinally and anticlinally. *C*, sweet orange fibrous root tip whose apical meristem has been terminated. The walls of whitish appearing cells are suberized or lignified. These cells pertain to the endodermis, hypodermis, epidermis, promeristem, and rootcap. *D*, dormant root tip. Cell walls of one layer of rootcap cells have become suberized. (*A*, ×670; *B,D*, ×300; *C*, ×250.)

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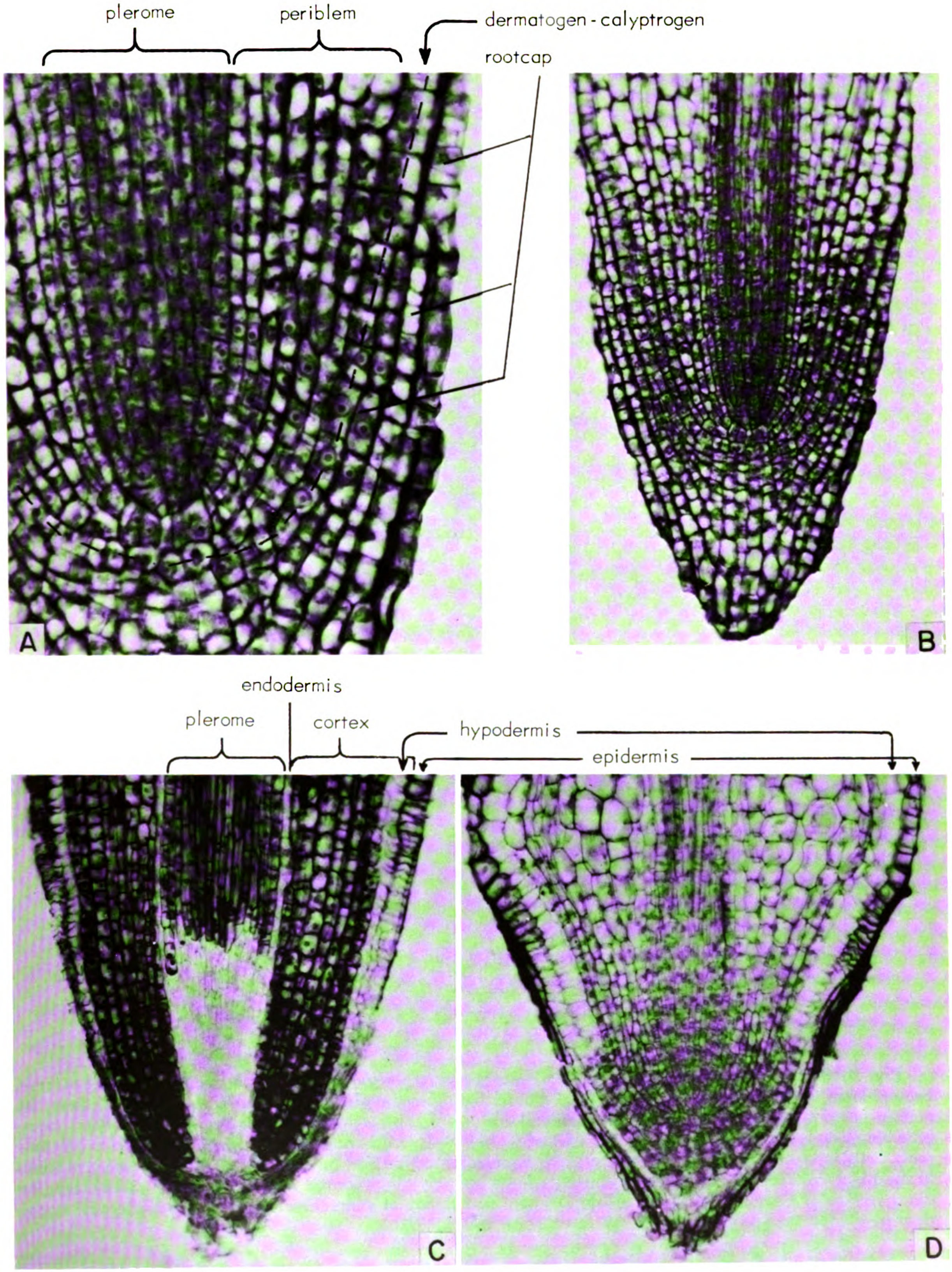
plerome
periblem
dermatogen - calyptrogen
rootcap
A
B
endodermis
plerome
cortex
hypodermis
epidermis
C
D

Fig. 1–16. *A*, cross-section through the post meristematic portion of a pentarch root tip of Mexican lime. Protophloem sieve tubes (white arrows) are differentiating in the phloem procambiumal strands. Xylem formation has not yet begun. *B*, longitudinal radial section of fibrous root of sweet orange. The distal sieve-tube elements are not yet mature (lower part of section). *C*, cross-section in region of elongation of lime root. Cells have enlarged and are thin-walled. Cytoplasm is thin and parietal. There are mature sieve tubes in the phloem strands, but tracheary elements have not yet differentiated in the xylem strands. *D*, longitudinal section of sweet orange postmeristem region and region of elongation. Details: *h*, hypodermis; *e*, epidermis; *c*, rootcap. (*A*, ×250; *B*, ×1,250; *C*, ×1,000; *D*, ×250.)

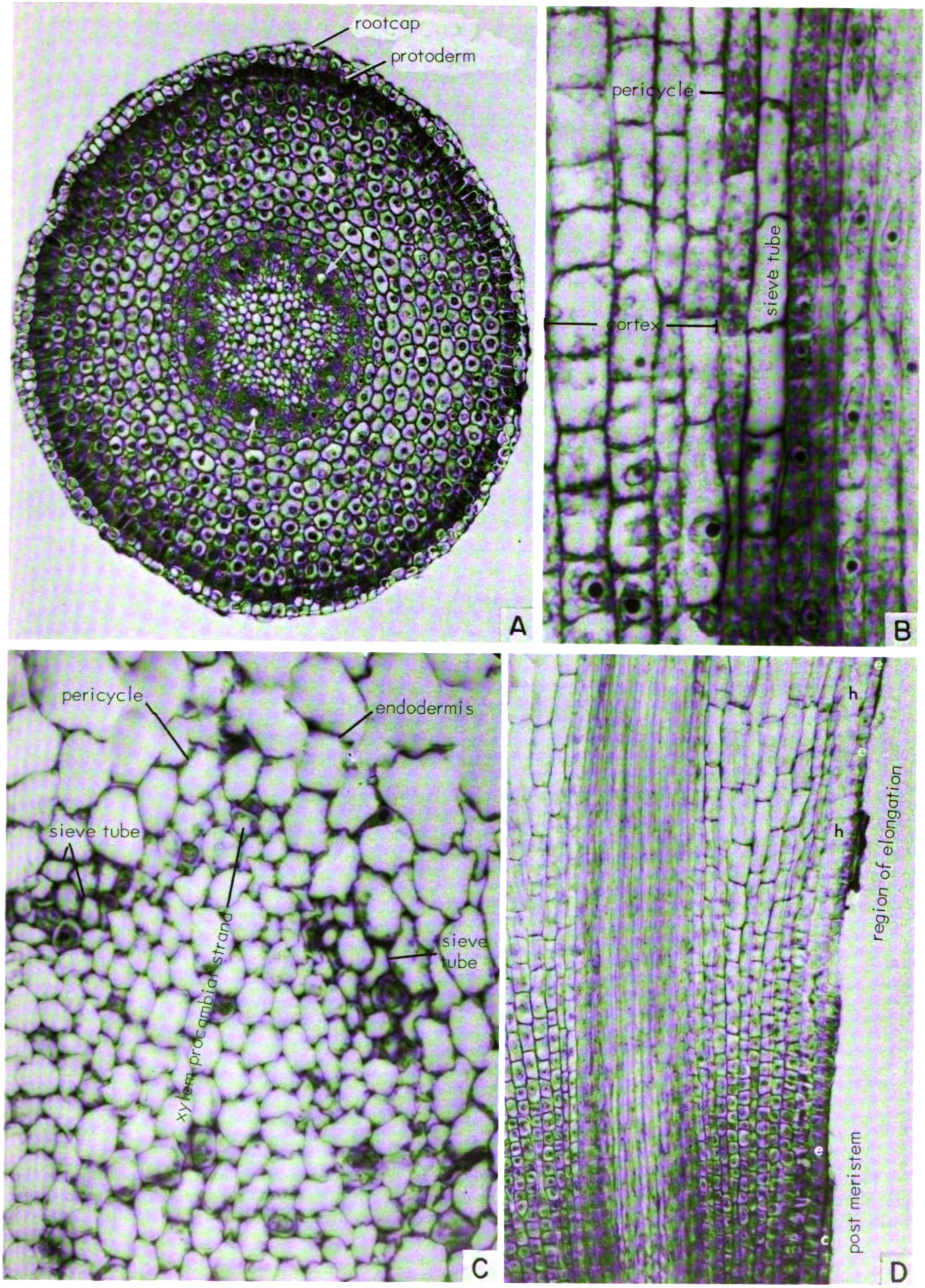
rootcap
protoderm
A
pericycle
sieve tube
cortex
B
pericycle
endodermis
sieve tube
xylem procambial strand
sieve tube
C
e
h
e
h
region of elongation
e
c
post meristem
D

Fig. 1–17. *A*, radial section of Pera sweet orange fibrous root grown in sand, loam, peat-moss mix in the greenhouse. *B*, radial section through the cortex and primary xylem strand of young sweet orange root. Outer walls of some epidermal cells have deteriorated. *C*, schematic drawing of citrus root. (*A*, ×250; *B*, ×500.)

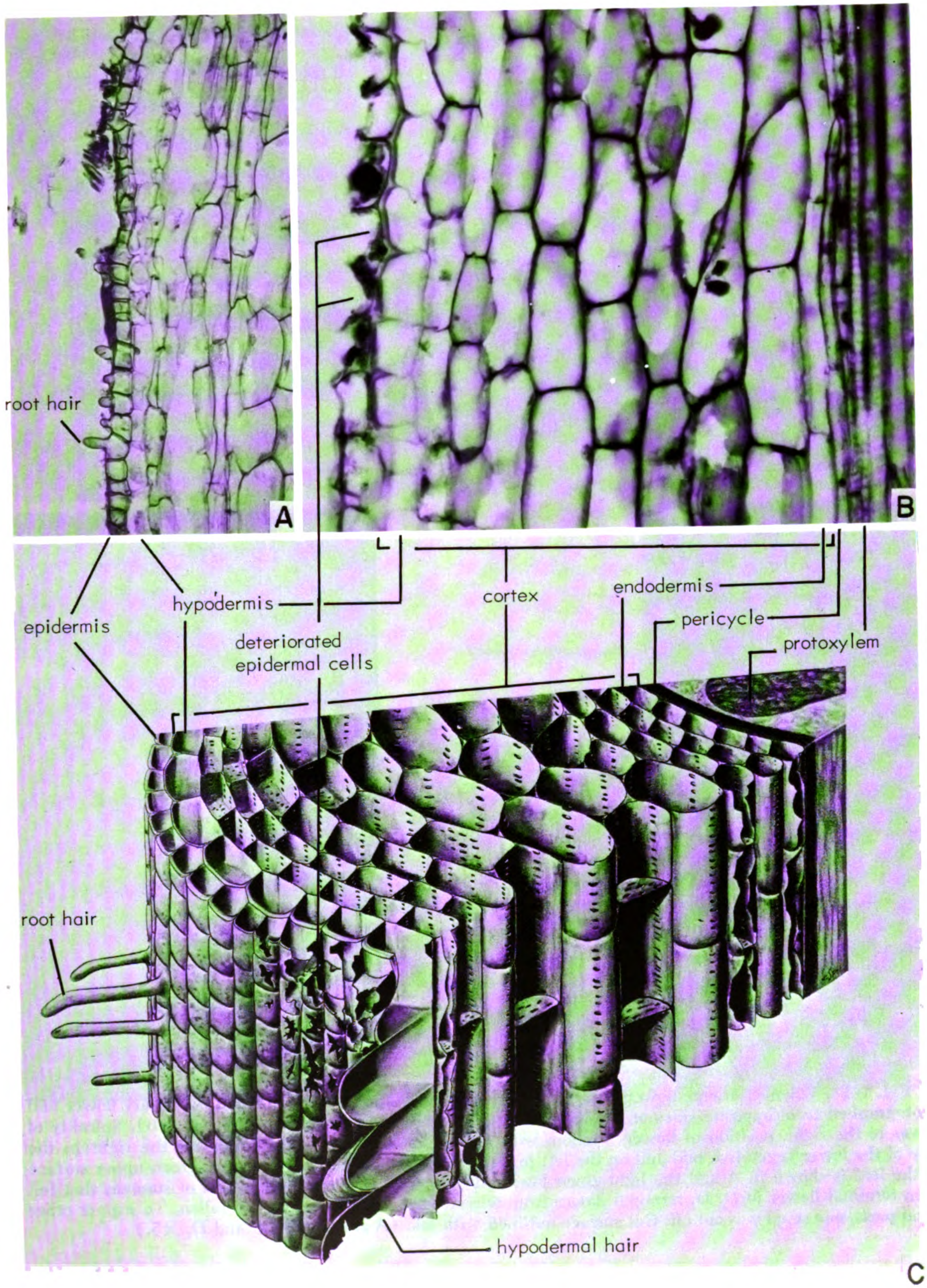
A
B
C
root hair
epidermis
hypodermis
deteriorated epidermal cells
cortex
endodermis
pericycle
protoxylem
root hair
hypodermal hair

Fig. 1–18. Valencia orange flowers and floral parts collected April 20, 1965. *A* and *B*, axillary flower bud photographed from opposite sides of the branch. Vegetative buds occur on one side only (*B*). Spirality of leaves to the right, position of flowers random with respect to vegetative bud, being on the right in the case of the lower vegetative bud and on the left in the case of the upper one. The dark-green upper surface of the leaf is shown in *A* and the light-green lower surface in *B*. *C*, part of the whorl of stamens that fell from terminal flower in *D*. *D*, terminal flower from which petals and stamens have fallen. To expose other floral parts, one sepal was cut off. Cut surface outlined with ink. (*A* and *B*, ×4; *C* and *D*, ×5.)

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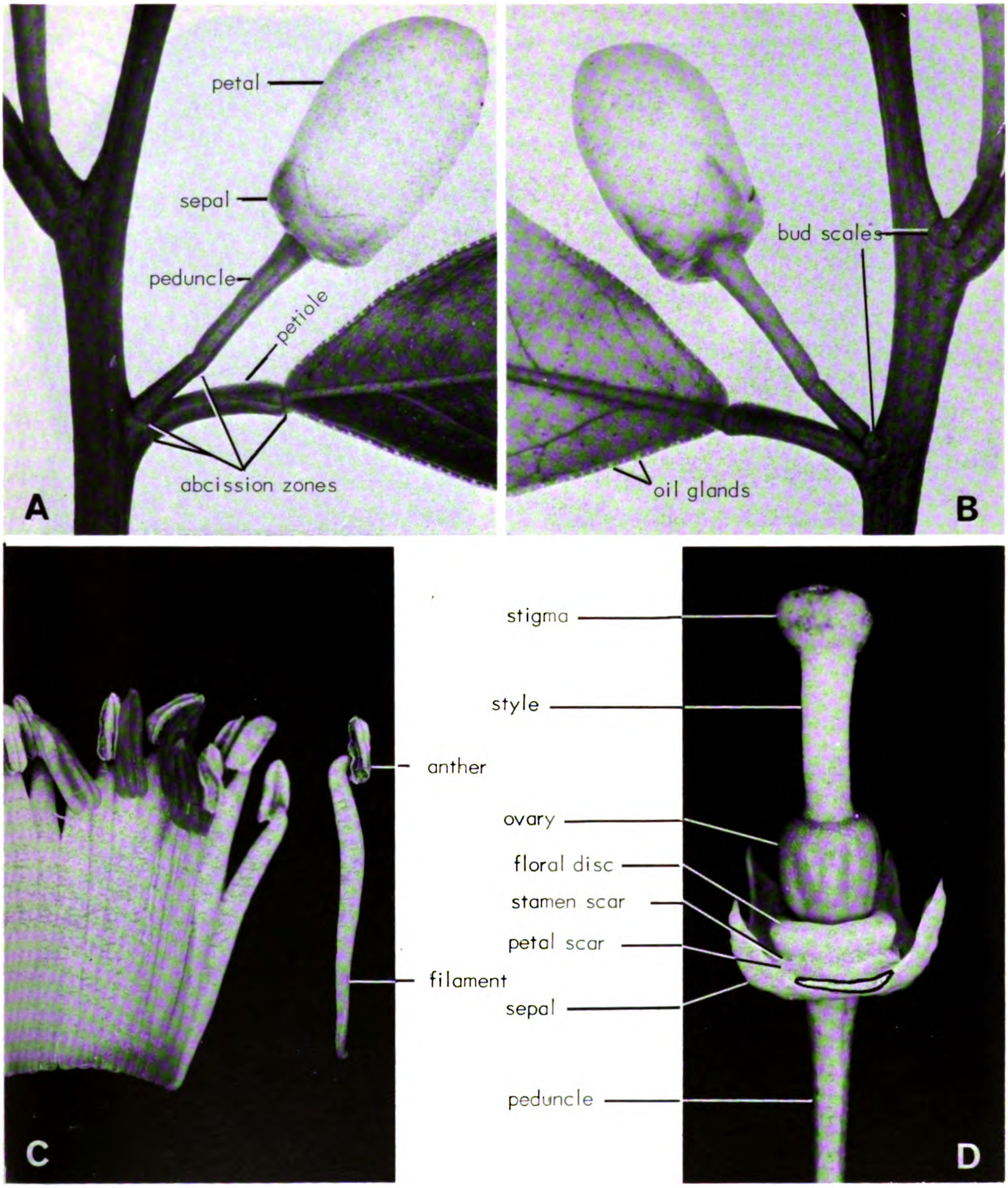
petal
sepal
peduncle
petiole
abcission zones
A
bud scales
oil glands
B
anther
filament
C
stigma
style
ovary
floral disc
stamen scar
petal scar
sepal
peduncle
D

Fig. 1–19. Longitudinal sections of flower buds of Mexican lime. *A*, flower whose petals have not yet opened. *B*, thick median section of portion of flower whose unopened petals were removed. Cleared and stained with safranin to demonstrate the vascular bundles. *C*, median section of the small axillary flower shown in *D*. Carpel primordia (*c*) and stamen primordia (*s*) are in early stages of development. Floral meristem (*f*) has not yet begun to elongate, *D*, median section of a flowering shoot with a large terminal flower and the younger axillary one shown in *C*. (*A*, ×16; *B*, ×10; *C*, ×90; *D*, ×11.)

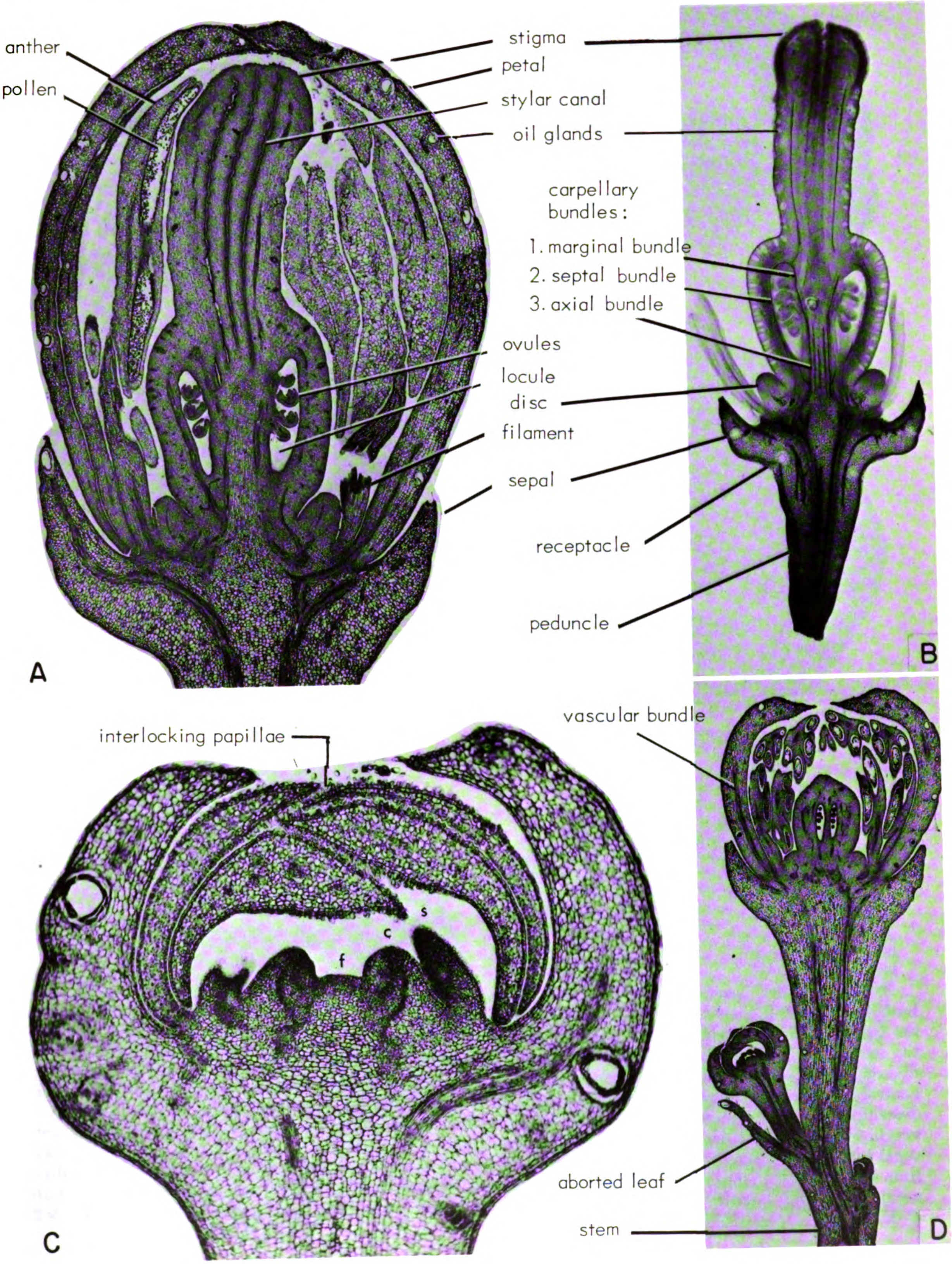
anther
pollen
stigma
petal
stylar canal
oil glands
carpellary bundles:
1. marginal bundle
2. septal bundle
3. axial bundle
ovules
locule
disc
filament
sepal
receptacle
peduncle
A
B
interlocking papillae
s
c
f
C
vascular bundle
aborted leaf
stem
D

Fig. 1–20. Cross-sections of a Mexican lime terminal flower bud which was collected from an axillary branch just after it erupted from the bud scales. Carpels and stamens are still in primordial stages. *A*, through the tip of the floral meristem, the more-or-less free ends of carpel primordia, the petals, and the sepals. *B*, the section immediately below *A*. Except at their tips, the wings of the lamina of carpel primordia are undiverged from the lamina of the adjacent primordia and the margins of the lamina are undiverged from the central axis. Locules result. *C*, through upper part of the receptacle where the whorl of staminal traces are diverging from the vascular cylinder. *D*, through the receptacle where the median petal traces are diverging from the vascular cylinder, and 100 microns above *E*. Details: *L*, lateral sepal bundle; *m*, median sepal bundle. *E*, through the receptacle where the whorl of sepal (*s*) and the petal-sepal (*ps*) traces diverge from the vascular cylinder. The petal-sepal traces are immediately below the petal traces shown in *E*. Lateral petal (*Lp*) and lateral sepal (*Ls*) traces have diverged from the sepal traces. (*A*, ×100; *B*, ×250; *C–E*, ×80.)

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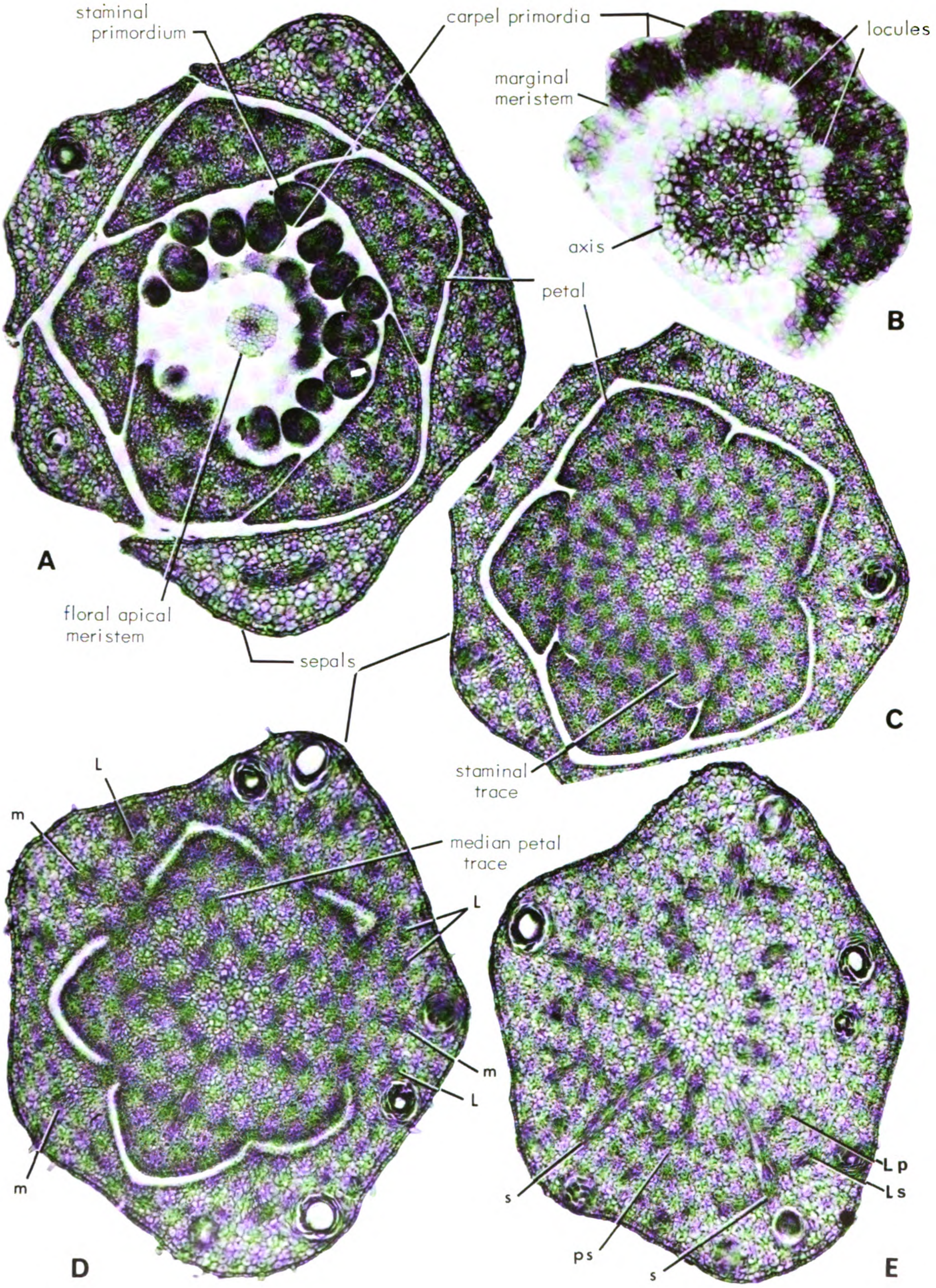
staminal primordium
carpel primordia
locules
marginal meristem
axis
B
petal
A
floral apical meristem
sepals
C
staminal trace
L
m
median petal trace
L
m
L
m
D
Lp
Ls
s
ps
s
E

Fig. 1–21. *A*, higher magnification of the median longitudinal section of Mexican lime ovary shown in the terminal unopened flower of figure 1–19, *A*. *B–D*, cross-sections of a lime flower with unopened petals. *B*, through the petals, anthers, and style. *C*, through the anthers, filaments, ovary at ovules, petals, and sepals. *D*, whorl of staminal traces in receptacle diverging from the vascular cylinder. Observation of adjacent sections indicates that the traces may remain unbranched and supply one stamen or be branched two or three times and supply two or three stamens. (*A*, ×115; *B*, ×28; *C*, ×24; *D*, ×28.)

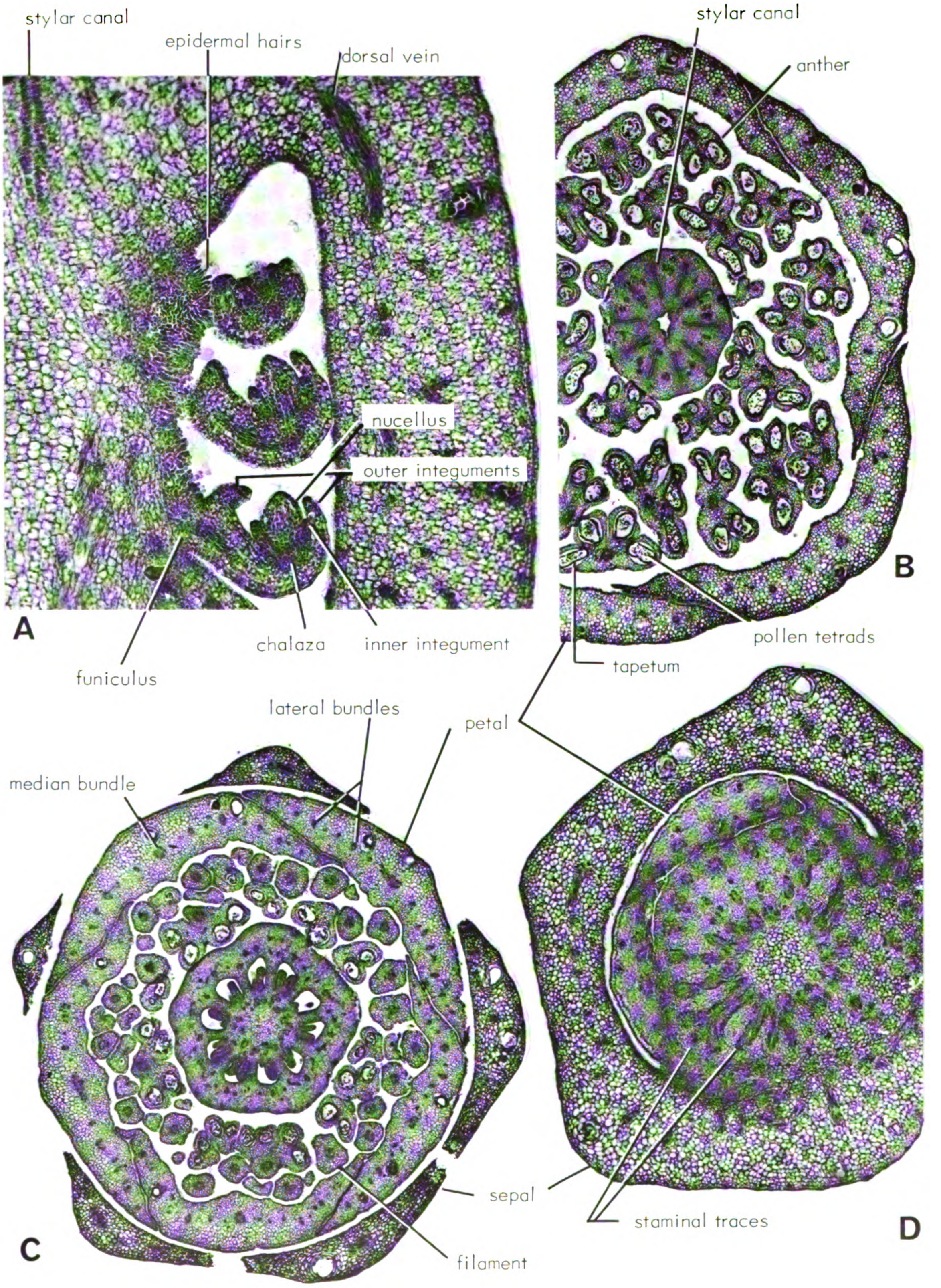
stylar canal
epidermal hairs
dorsal vein
nucellus
outer integuments
A
chalaza
inner integument
funiculus
stylar canal
anther
B
pollen tetrads
tapetum
lateral bundles
petal
median bundle
sepal
staminal traces
D
C
filament

Fig. 1–22. Cross-sections of ovaries of Valencia orange in various stages of development. *A*, from axillary flower collected April 16, 1965, whose petals had not yet opened. Ovules fairly well formed. *B*, from terminal fruit collected April 26, 1965, whose style had fallen. A few juice sacs have been initiated; epidermal hairs have appeared in the stylar canals. Details: *i*, inner integument; *m*, micropyle; *n*, nucellus; *o*, outer integument. *C*, from terminal fruit collected May 12, 1965. Section near the top of the locules where the axial vascular bundles have diverged from the axis into the septa to become marginal bundles. The juice sacs have elongated, and mucilage (*m*) has appeared in the locules. *D*, from fruit collected July 9, 1965. Pericarp has enlarged greatly by cell division, but locules are still relatively small. The sac portion of the juice sacs is beginning to form on the stalks. One seed has begun to form, its nucellus is greatly enlarged, and its vascular bundle (arrow) may be seen in cross-section. The section is from the lower half of the fruit where the vascular bundles in the core are in an axial position. See figure 1–23, *C* for higher magnification of juice sacs. (*A*, ×120; *B*, ×58; *C*, ×41; *D*, ×12.)

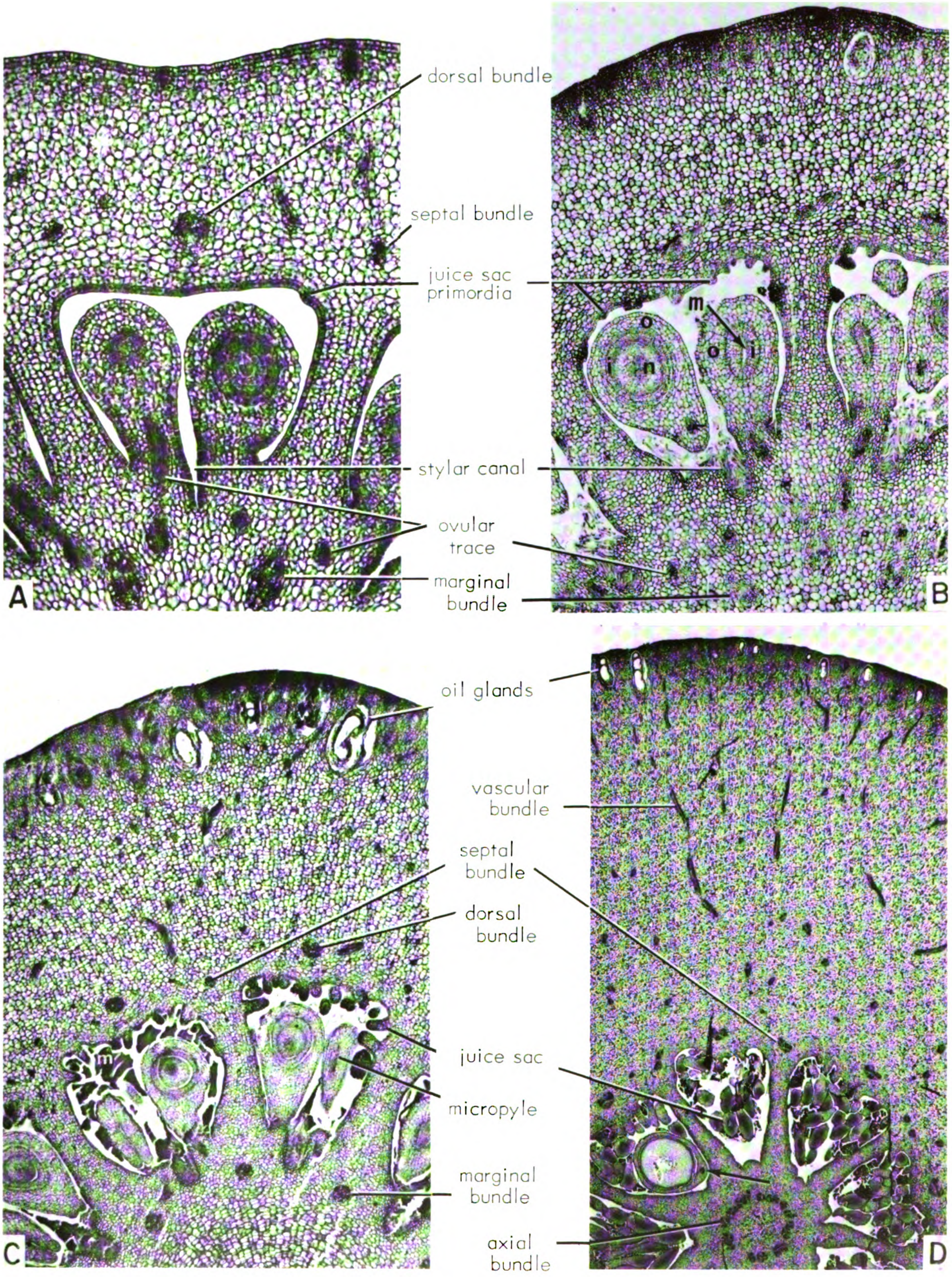
dorsal bundle
septal bundle
juice sac primordia
m
o
i
n
o
i
stylar canal
ovular trace
marginal bundle
A
B
oil glands
vascular bundle
septal bundle
dorsal bundle
juice sac
micropyle
m
marginal bundle
axial bundle
C
D

Fig. 1–23. Cross-sections of ovaries of Valencia orange. *A1* and *A2*, cross-sections of pericarp and locules, respectively, of a sweet orange fruit in Stage II of development, collected on July 27, 1965. Locules have become as large as the pericarps in their radial dimension. The mesocarp cells have begun to differentiate, and air spaces are present. Parenchyma cells have differentiated at the center of the juice sacs. *B*, carpellary emergences on a septum of the ovary of a terminal fruit collected May 12, 1965. Section from same fruit as in figure 1–22, *C*. Some cells (*c*) have become detached from the emergences. *C*, locule from a fruit in which the sac portion of the juice sacs are meristematic and in an earlier stage of development than *A2*. Sacs are in oblique section. Same ovary as figure 1–22, *D*. (*A1, A2*, ×8; *B*, ×250; *C*, ×50.)

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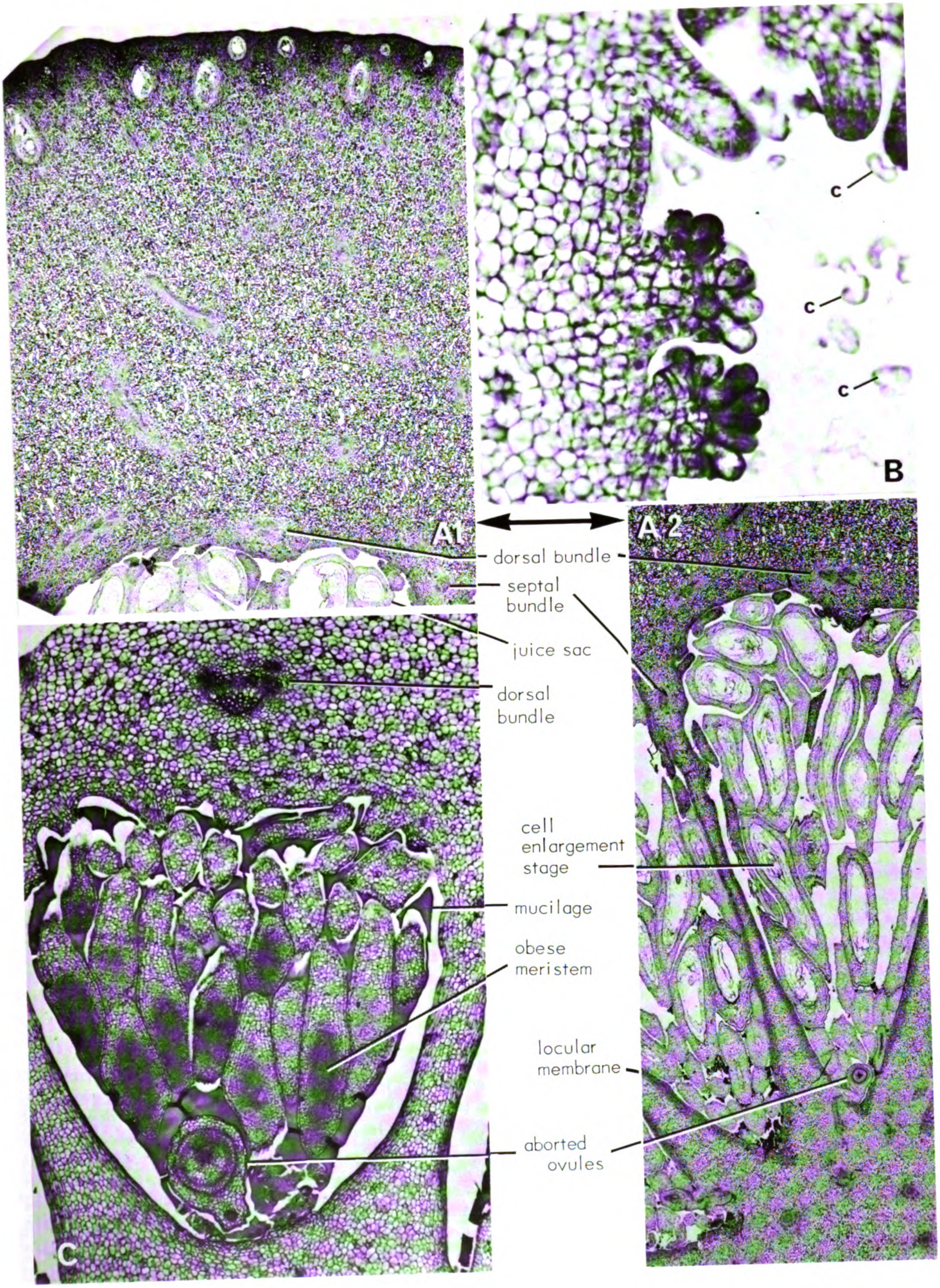

c
c
c
B
A1
A2
dorsal bundle
septal bundle
juice sac
dorsal bundle
cell enlargement stage
mucilage
obese meristem
locular membrane
aborted ovules
C

Fig. 1–24. Sections of endocarps of Valencia orange ovaries with juice sac in early stages of formation. ***A–C*, from terminal flowers collected April 20, 1965,** whose petals and stamens had fallen as in figure 1–18, ***D. A*, recent anticlinal divisions in epidermal cells** and periclinal division in subepidermal cells. *B*, **bulge at left caused by divisions and enlargements of subepidermal cells. In bulge at right, recent oblique and periclinal divisions in epidermis of juice sac primordium.** *C*, random divisions in epidermis and subepidermal cells. ***D–G*, from ovaries of terminal flowers** collected April 26, 1965. Styles have fallen. *D*, periclinal **and anticlinal divisions in epidermis of young juice sac.** *E–G*, random divisions in epidermal and subepidermal **cells of juice sac primordium.** *H*, **locule of ovary of a terminal flower** collected May 12, 1965. **Juice sacs have elongated and meristem is apical. One carpellary emergence** (*ce*) **is present.** Ovules have mature mega**spores.** (***A–C*, ×1,100; *D–G*, ×1,300; *H*, ×100.**)

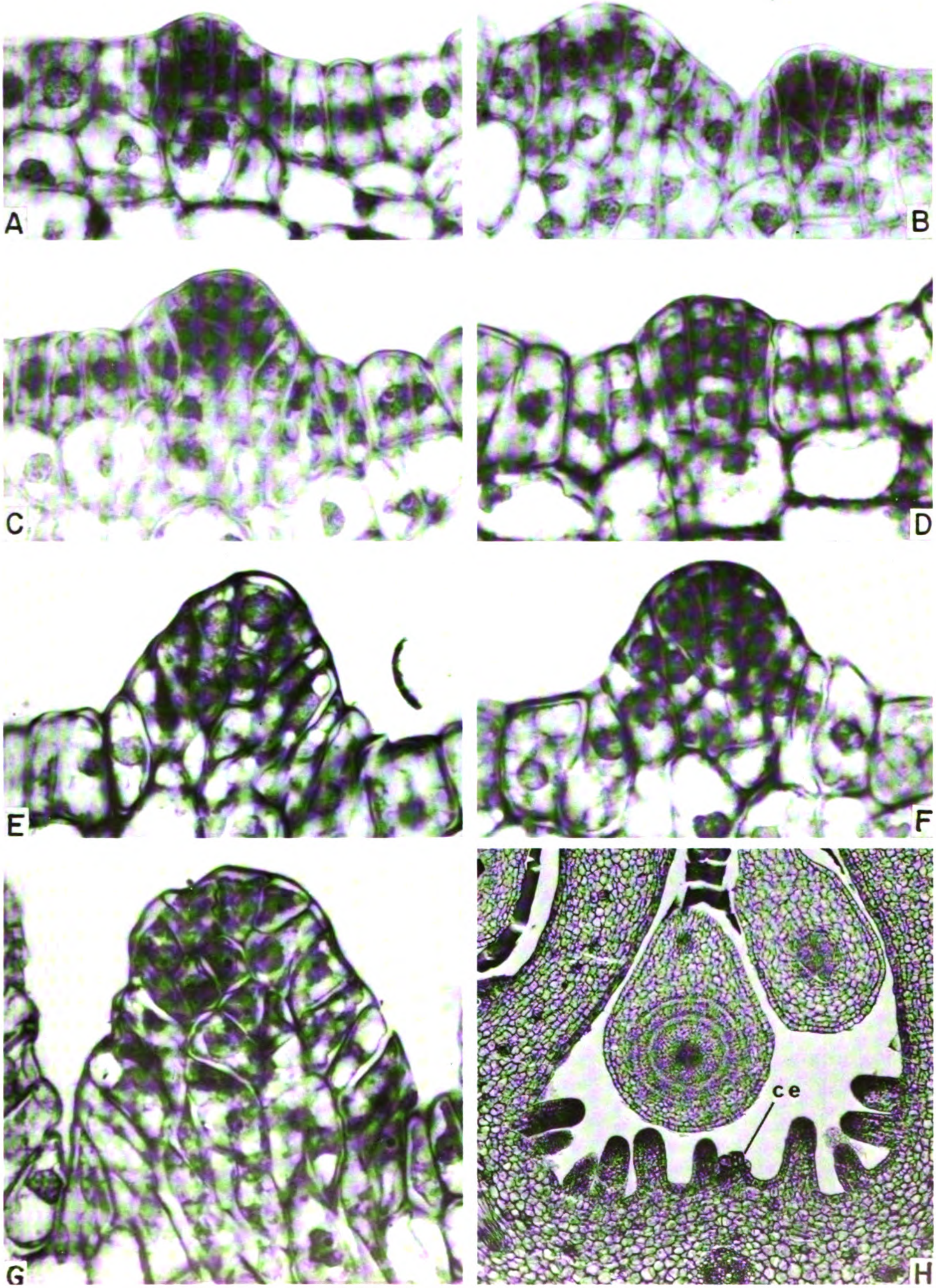
A
B
C
D
E
F
G
ce
H

Fig. 1–25. *A–D,* **drawings and photomicrographs of Valencia orange fruit.** *A,* **diagramatic drawing of mature fruit featuring the location of the main vascular bundles (septal, dorsal, axial, ovular, seed, and marginal).** *B,* **diagrammatic drawing of the ovules as they appeared on June 1, 1965, about six weeks after the petals opened. Drawing of lower ovule is after the section pictured in** *D* **and the series of sections adjacent to it. Upper ovule is a photomicrograph of a whole ovule that was cleared and then stained with safranin.** *C,* **epidermal hairs in the stylar canal that may secrete part of the mucilaginous material (*m*) found in the locules.** *D,* **oblique-longitudinal section of ovule of fruit collected June 1, 1965. The functional megaspore (embryo sac) is at the middle of the nucellus. Compare with figure 1–26,*A*. Details: *c*, chalaza; *f*, funiculus; *i*, inner integument; *m*, mucilage; *o*, outer integument; *v*, vascular bundle. (*B*, ×100; *C*, ×600; *D*, ×115.)**

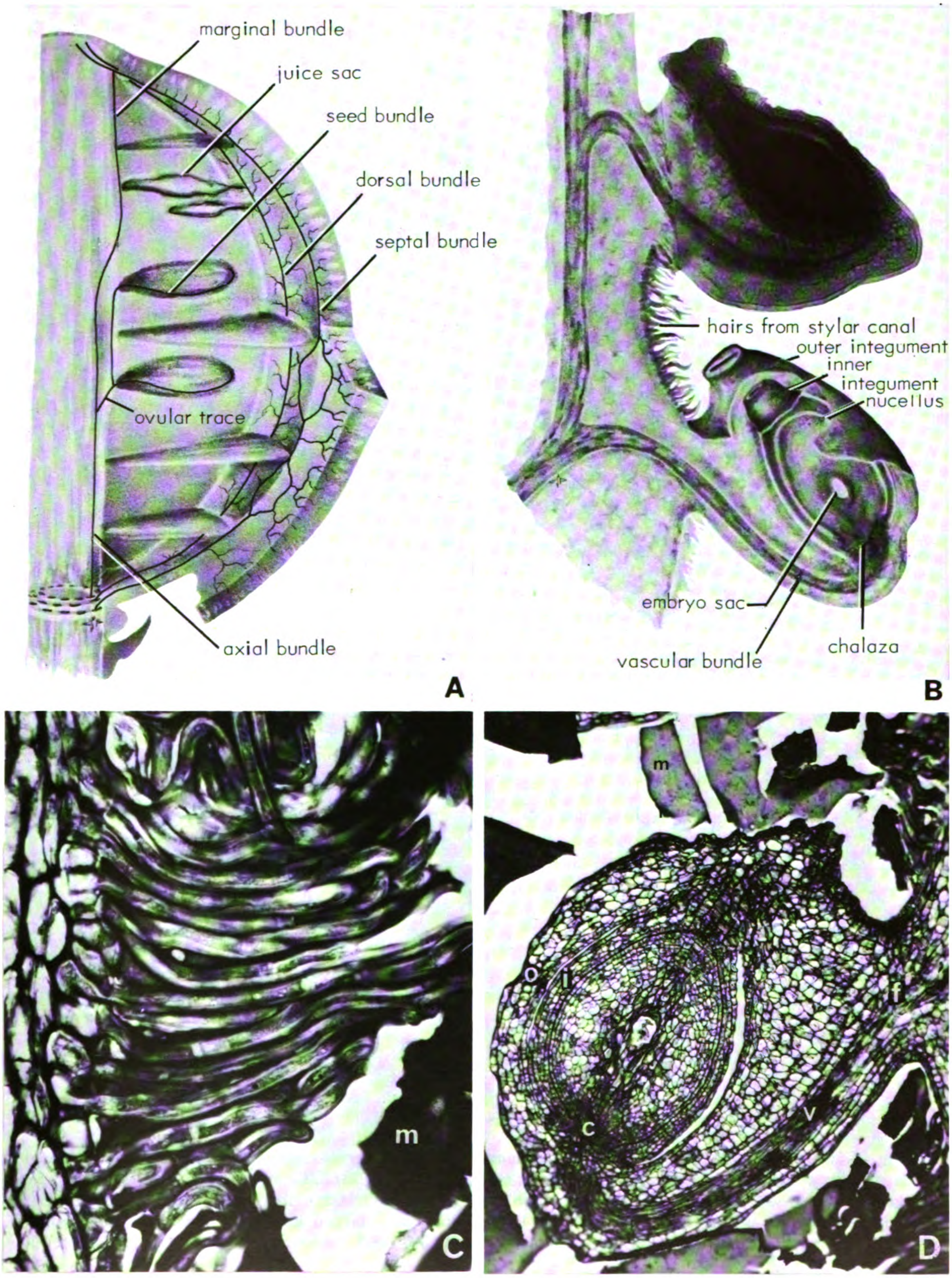
marginal bundle
juice sac
seed bundle
dorsal bundle
septal bundle
ovular trace
axial bundle
A
hairs from stylar canal
outer integument
inner integument
nucellus
embryo sac
vascular bundle
chalaza
B
m
C
m
o
i
f
c
v
D

Fig. 1–26. Stages in seed development of Valencia orange. *A*, cross-section of ovules through functional megaspore or embryo sac (see arrow) of a fruit collected June 1, 1965. Compare with longitudinal sections in figure 1–25, *B* and *D*. *B*, cross-section of ovule through multinuclear embryo sac (arrow) collected July 9, 1965 (same fruit as shown in figure 1–22,*D*). Nucellus has enlarged greatly (*s*, seed vascular bundle). *C* and *D*, cross-sections of a developing seed collected July 30, 1965. (*A*, ×150; *B*, ×40; *C*, ×26; *D*, ×160.)

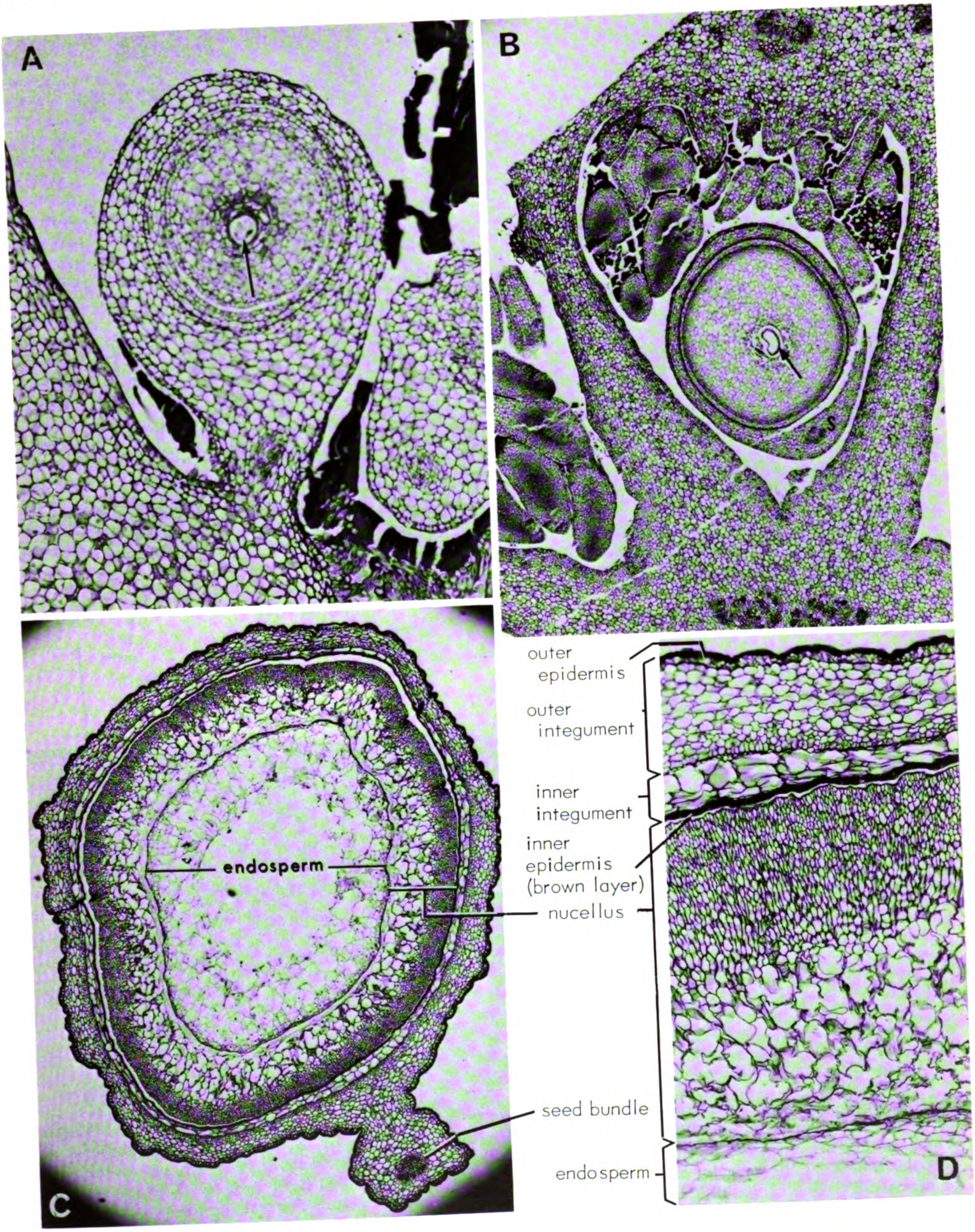
A
B
S
outer epidermis
outer integument
inner integument
inner epidermis (brown layer)
endosperm
nucellus
seed bundle
endosperm
C
D

Fig. 1–27. Longitudinal sections of immature seeds of Valencia orange. *A*, *B*, and *D*, collected August 26, 1965, and *C*, on July 30, 1965. *A*, micropylar end with young embryos. *B*, somewhat older embryos in which cotyledons and procambial strands have formed. *C*, chalazal end of seed. The terminus of the seed vascular bundle at the vascular cap is in cross-section. Plane of section is at right angle to *D*. There is a cap of brown cells which separates the nucellus from the cap of vascular tissue. The phloem lies adjacent to the layer of brown cells. *D*, whole seed with the vascular bundle throughout most of its length in the plane of the section. (*A*, ×60; *B*, ×28; *C*, ×24; *D*, ×20.)

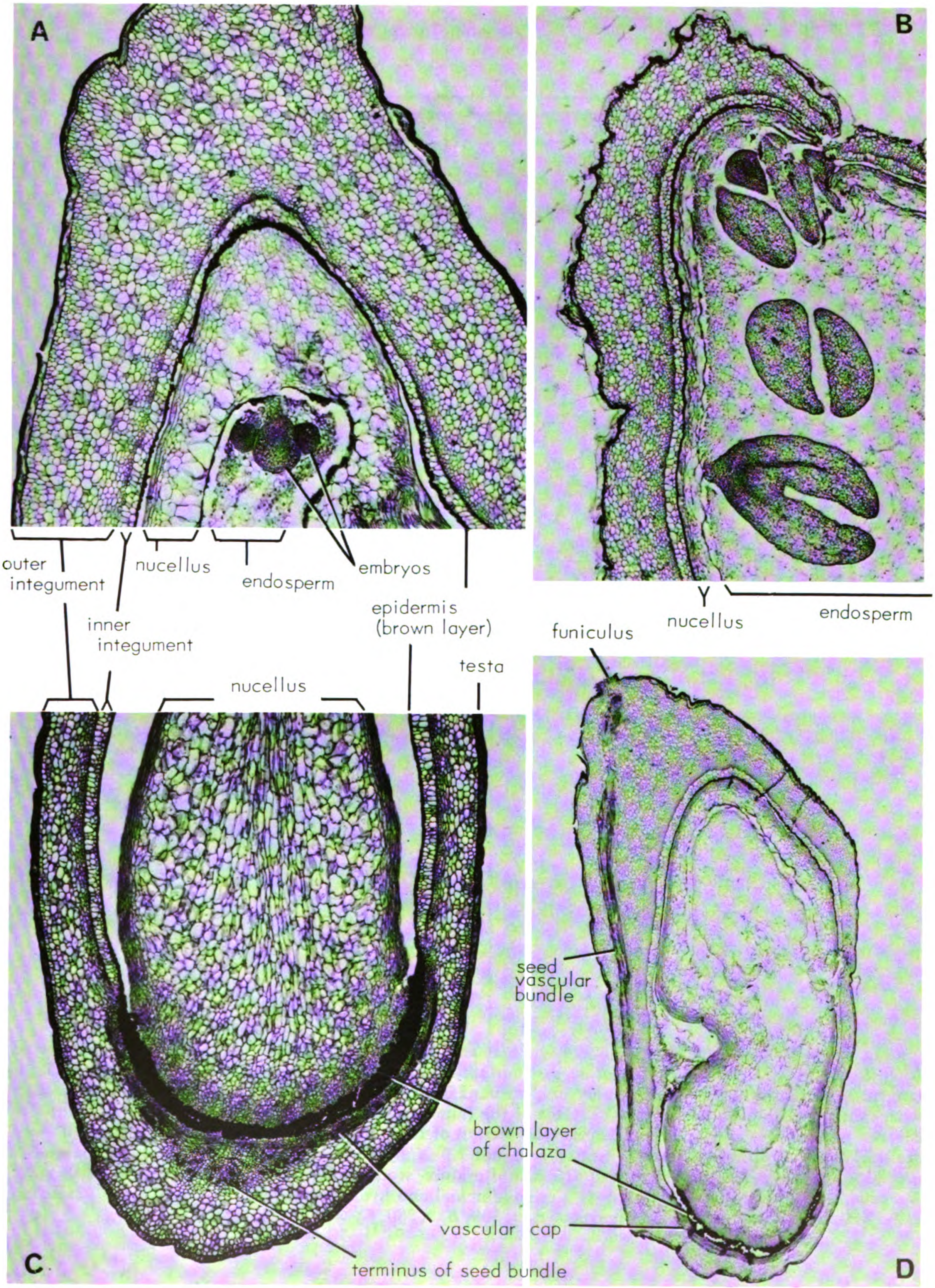

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Fig. 1–28. Sections of Valencia orange seeds. *A* and *B*, sections of seeds from 1965 bloom collected August 26, 1965. *D* and *E*, sections of seeds from 1964 bloom collected when ripe on June 1, 1965. *A*, tangential section of brown layer (inner epidermis of inner integument). *B*, radial sections of integuments where they are attached to the chalaza. Cells of the immature testa are much elongated, thin-walled, and covered with a layer of mucilage. *C*, tangential section of testa of mature Valencia orange seed. Cell walls are thickened and lignified. Sections treated with hot 1.0 N hydrochloric acid after sectioning to remove mucilage. Stained with safranin. *D*, cross-section of mature seed stained with safranin and haematoxylin. *E*, radial section of seed coats treated with phloroglucinal and hydrochloric acid. Lignified cells of testa became red and mucilage remained colorless. (*A*, ×300; *B*, ×280; *C*, ×300; *D*, ×260; *E*, ×180.)

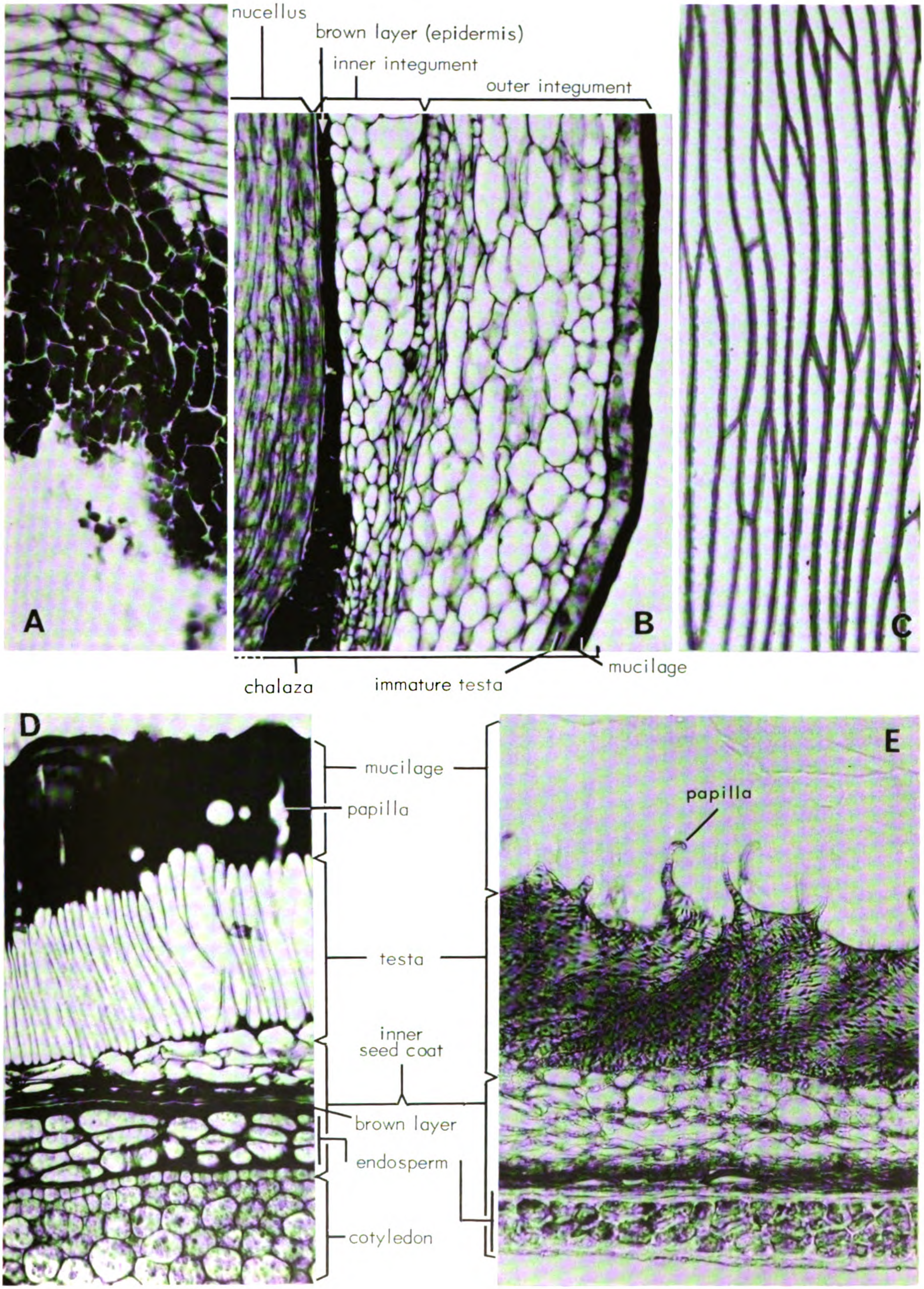
nucellus
brown layer (epidermis)
inner integument
outer integument
A
B
C
chalaza
immature testa
mucilage
D
E
mucilage
papilla
papilla
testa
inner seed coat
brown layer
endosperm
cotyledon

Fig. 1–29. Sections of pericarp of Valencia orange. *A*, exocarp. Cuticle intrudes between epidermal cells. Subepidermal cells have thick layers of cytoplasm containing chromoplasts (arrows). *B*, albedo cells in section showing protruding arms that join adjacent cells (arrows). *C*, albedo cells from macerated tissue with arms extending from the bodies of the cells. *D*, endocarp. Epidermis (*e*) and tightly packed and stretched parenchyma (below). Mesocarp (above). *E*, chloroplast from immature green fruit. Arrows indicate the small globules along the edge of the grana. *F*, chromoplast in ripe fruit that was transformed from a chloroplast. Grana have degenerated and osmiophilic globules have increased in size. Details: *f*, frets; *g*, grana; *L*, large osmiophilic globules; *m*, mitochondria; *st*, starch; *s*, stroma. (*A*, ×1,250; *B*, ×130; *C*, *D*, ×180; *E*, ×32,000; *F*, ×23,000. *E* and *F* courtesy of W. W. Thomson.)

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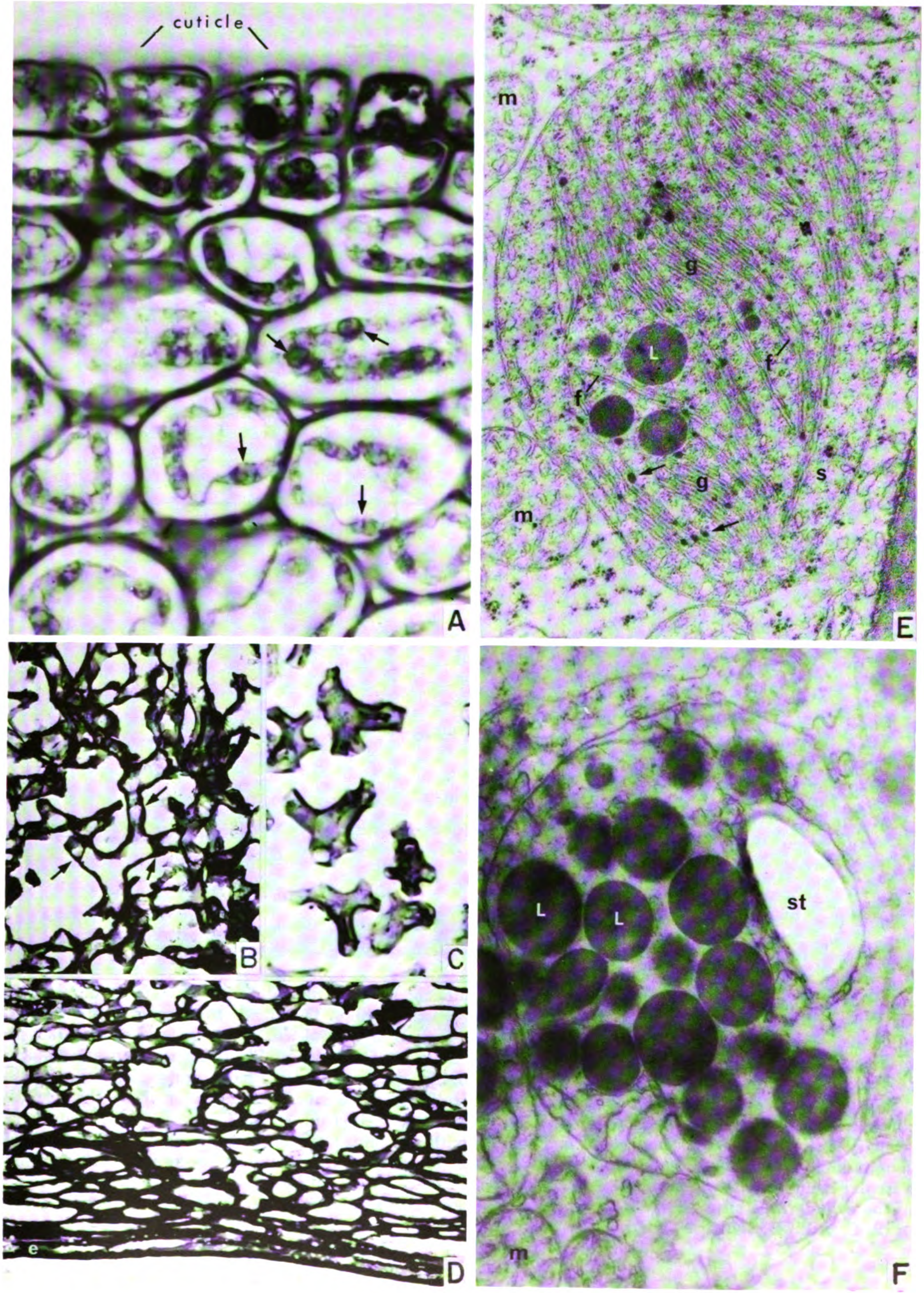
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Fig. 1–30. *A*, *B*, *C*, and *E*, sections of mature Valencia orange fruit. *A*, cross-section of the pericarp, the outer portion of two locules, and a septum between the locules. Oil glands that are in median section abut the epidermis and are of various sizes. *B*, cross-section of central axis near the base of the fruit and of inner part of locules. There is mesocarp-like tissue centripetal to endocarp-like tissue which occurs adjacent to the locules. In addition to the juice sacs (*j*), gall-like outgrowths (*g*), have grown into the locules. Vascular bundles are axial. *C*, tangential sections of fruit with juice sacs in cross-section. Note their cellular nature. The stalks have no vascular bundles. *D*, tracheid-like cells from stalk of juice sac of shaddock (*C. grandis* [L.] Osbeck); stalk macerated and stained with safranin. Cells occur separately or in packets. *E*, longitudinal section of peduncle end of fruit. Vascular bundles of the peduncle receptacle region form a vascular cylinder with considerable secondary tissue. Dorsal and axial bundles diverge from the distal end of the cylinder. Details: *f*, floral disc; *g*, gall-like outgrowths; *j*, juice sac; *s*, sepal; *t*, tear in section. (*A*, *B*, ×12; *C*, ×32; *D*, ×215; *E*, ×8.)

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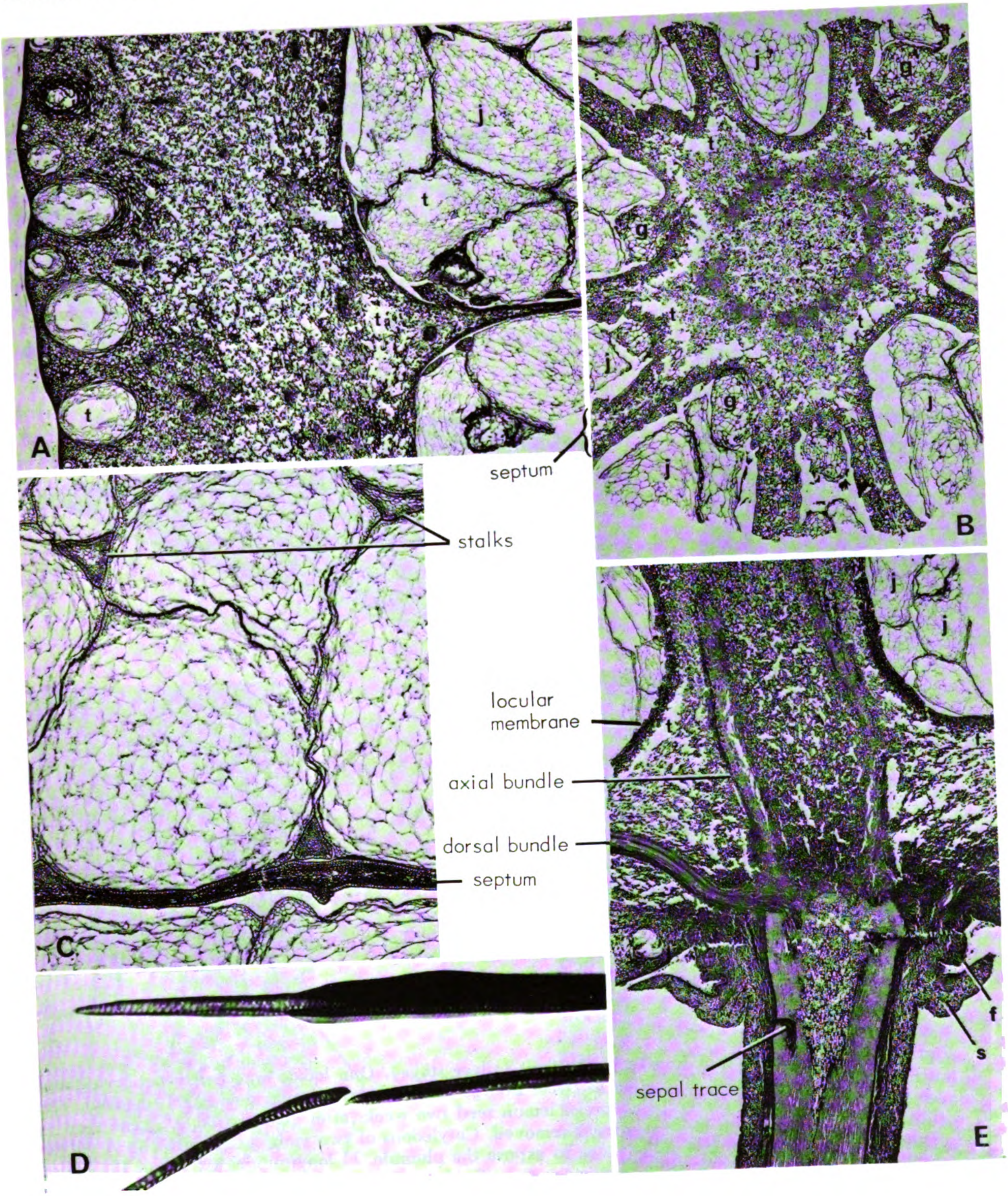
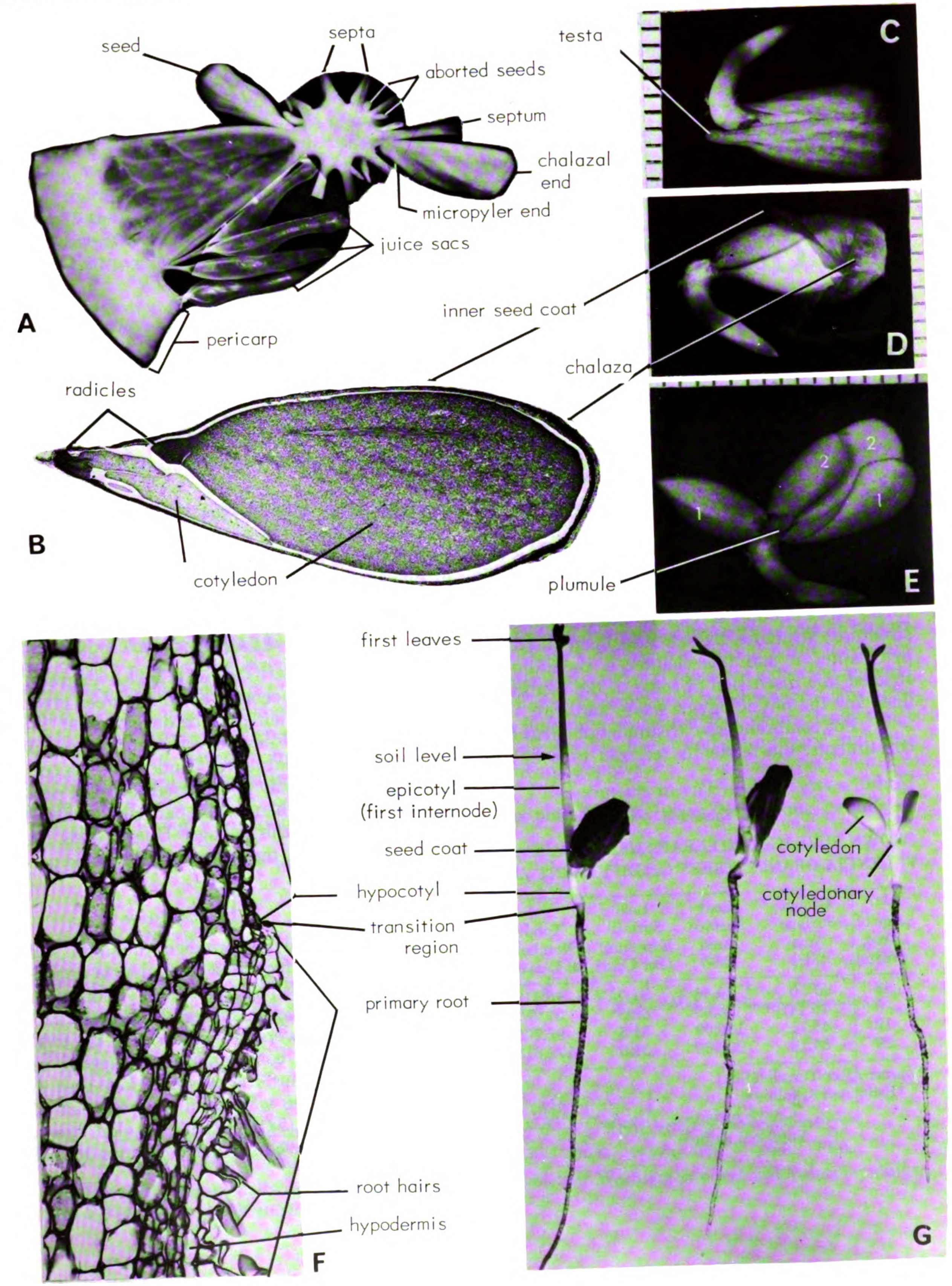

A
t
j
g
septum
B
stalks
C
septum
D
locular membrane
axial bundle
dorsal bundle
sepal trace
f
s
E

Fig. 1–31. *A*, cross-section of Eureka lemon fruit (silver stage). One locule was partly cut away, and all but four juice sacs removed. *B*, section of sweet orange seed with testa (outer seed coat) removed. The radicles of two embryos are present. *C*, Eureka lemon seed two weeks after germination. *D*, same seed as *C* with testa removed. *E*, with inner seed coat removed. Cotyledons of two embryos are present. A cotyledon of the embryo numbered 1 is pulled back to expose the plumule. *F*, longitudinal section of the cortex and epidermis of sweet orange at the transition region. Epidermal cells of the hypocotyl are small, and without hairs but with a cuticle. Below the transition region the cell walls are suberized or lignified and stained red with safranin. Root hairs and a hypodermis are present. *G*, seedlings of sweet orange. Cotyledons remain underground and usually within the seed coat. For the seedling on the right, the seed coat was removed and cotyledons spread apart. The roots are brownish, whereas the hypocotyl and the lower portion of the epicotyl are white. (*A*, ×2.2; *B*, ×8; *C–E*, ×3; *F*, ×150; *G*, ×1.)


seed
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micropyler end
juice sacs
pericarp
A
testa
C
inner seed coat
D
chalaza
radicles
B
cotyledon
2
2
1
1
plumule
E
first leaves
soil level
epicotyl (first internode)
seed coat
hypocotyl
transition region
primary root
root hairs
hypodermis
F
cotyledon
cotyledonary node
G

CHAPTER 2

The General Physiology of Citrus

LOUIS C. ERICKSON

PHYSIOLOGY, THAT PORTION of biological knowledge which deals with function, is so intimately related to other branches of biology that it is difficult to bring all aspects of the physiology of citrus into one chapter, and no effort will be made to do so. Function is closely interrelated with structure. Structure has already been discussed in Chapter 1, and this chapter is therefore restricted to a discussion of some of the factors influencing the physiology of leaves and fruit.

For comprehensive treatment of citrus fruit constituents, the reader is referred to books by Sinclair (1961), Kefford (1959), and Bartholomew and Sinclair (1951). In addition, the chemistry and technology of citrus and its products are covered in two works: Braverman (1949) and U. S. Department of Agriculture (1962).

Table 2–1

CROWN SURFACE AND LEAF SURFACE AREA OF VALENCIA ORANGE TREES OF DIFFERENT AGES

Age of Tree, (Years)	Crown Surface Area (M^2)	Leaf Surface Area (M^2)	Leaf Surface Exposure* (Per Cent)	Total Number of Leaves
3	17.56	34.44	51.0	16,419
6	19.14	58.98	32.5	32,257
12	45.71	146.09	31.3	92,708
29	64.19	202.86	31.6	172,613

Source: After Turrell (1961).

*Estimated as follows:

$$\frac{\text{Crown surface}}{\text{Total leaf surface}} \times 100.$$

LEAVES

Although citrus varieties are evergreen, continual leaf replacement occurs as trees grow. Until trees reach their peak of development, the annual formation of new leaves normally exceeds the annual loss of old leaves. Under adverse conditions, this trend may be reversed for short periods: for example, when a wind partially defoliates trees or a freeze kills foliage. Unless trees are severely debilitated, they recover and resume normal growth.

Leaf Area

Turrell (1961) determined the total leaf area and crown surface area for Valencia orange trees *(Citrus sinensis* [L.] Osbeck) of various ages (see table 2–1). His data showed that crown surface areas of trees were far smaller than the total leaf areas, so that considerable overlapping of leaves occurred. Thus, the crown surface area in a three-year-old tree was equal to about half of the total leaf area (51 per cent). In older trees, crown areas were even smaller in relation to leaf area, representing about 31.3 to 32.5 per cent of total leaf area. If the total leaf areas for Valencia trees studied by Turrell were spread out over a flat surface, they would cover squares with sides equal to 5.87, 7.68, 12.09, and 14.24 meters (19.3, 25.2, 39.7, and 46.7 feet). With the possible exception of the three- and six-year-old trees, these areas represent more ground surface than is allotted to trees in an orchard. Thus, there are normally two sources of shading in orange orchards: leaves that overlap and trees that shade one another.

The extent to which light limitations retard the growth of trees or fruit is not known. However, Turrell (1961) showed that the minimum leaf area needed (2.3 m^2) to produce a kilogram of fruit is found in nine-year-old trees. One reason for decreased efficiency in older trees may well be the limitation of light. This factor will be considered further in the discussion of photosynthesis (see p. 91).

Leaves in the interior of a citrus tree tend to be flatter than exterior leaves. Monselise (1951*c*) found that 90 per cent of interior leaves of Shamouti orange were flat compared with 13 per cent of exterior leaves on a southern exposure and 44 per cent on a northern exposure. Marsh grapefruit had 86 per cent flat leaves in the interior, but in this variety more of the exterior leaves were flat than in the orange (i.e., 20 per cent on the southern and 60 per cent on the northern exposure).

Citrus leaves vary greatly in size. Turrell (1961) measured leaves on trees of various ages and found that size ranged from under 1.5 cm in blade length to as long as 18 cm (see table 2–2). The largest leaves were found on the youngest tree (three years of age). The most common blade length range was 6 to 7.5 cm for all trees except the oldest, where it was 4.5 to 6 cm.

Abscission

Orange leaves drop throughout the year (Wallace *et al.*, 1954; Erickson and Brannaman, 1960). However, the greatest leaf drop occurs during the spring flowering period, although other periods of accelerated drop do occur at various times (fig. 2–1). Scott, Schroeder, and Turrell (1948) found that leaf drop is most active during seasonal growth flushes. Shavit (1956) showed that a very marked abscission of green leaves occurs at about the beginning of the last week of blossoming. Leaves shed were of all sizes, including very young ones of the current season. There was also a shedding of shoot tips.

The age reached by an orange leaf before it falls is variable. Kelley and Cummins (1920) sampled leaves more than two years old. However, Wallace *et al.* (1954) found that most leaves on trees they studied had fallen by the time they were seventeen months old and almost all by twenty-four months.

Numerous environmental factors play a significant role in determining how long a leaf remains on the tree. The more common factors contributing to the early drop of citrus leaves are temperature, and soil moisture extremes, mineral nutrition problems, high wind velocity, low relative humidity, excessive root injury from fungi and nematodes, insect and mite injury, and misuse of some sprays.

Experiments in which water was withheld from potted citrus trees for various periods of

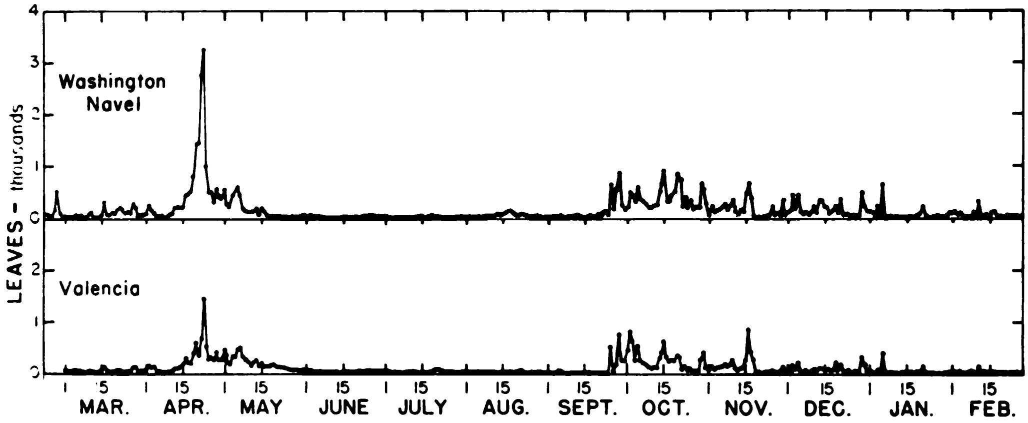

Fig. 2–1. Average daily drop of leaves from five trees each of Washington navel and Valencia orange, 1958–59. (Erickson and Brannaman, 1960.)

Table 2–2

NUMBER OF LEAVES, MEAN FRESH WEIGHTS, AND MEAN AREAS FOR VARIOUS LEAF-SIZE CLASSES BASED ON BLADE LENGTHS OF LEAVES FROM VALENCIA ORANGE TREES OF DIFFERENT AGES

	Blade Length Range (Cm)													
Characteristic	0–1.5	1.5–3.0	3.0–4.5	4.5–6.0	6.0–7.5	7.5–9.0	9.0–10.5	10.5–12.0	12.0–13.5	13.5–15.0	15.0–16.5	16.5–18.0	Total	Mean
3-Year-Old Tree														
Number of leaves	2	169	1,063	2,905	4,729	3,920	2,346	959	260	46	11	9	16,419	....
Mean weight per leaf (gm)	....	0.087	0.148	0.287	0.474	0.727	1.057	1.506	1.823	2.625	....	....	10.62 kg	....
Mean area per leaf (cm²)	....	2.70	5.12	9.28	15.05	23.09	34.34	48.76	62.50	85.42	110.0	129.5	34.44 m²	....
Weight per cm² of area (gm)	....	0.032	0.029	0.031	0.031	0.031	0.031	0.031	0.029	0.031	0.031	0.031	....	0.031
6-Year-Old Tree														
Number of leaves	5	419	3,100	9,298	13,070	8,174	2,607	503	73	8	0	0	37,257	....
Mean weight per leaf (gm)	....	0.063	0.14	0.26	0.42	0.68	0.99	1.31	1.88	....	0	0	17.30 kg	....
Mean area per leaf (cm²)	....	2.26	4.94	8.89	14.70	22.53	33.97	43.00	60.00	....	0	0	58.98 m²	....
Weight per cm² of area (gm)	....	0.028	0.028	0.029	0.029	0.030	0.029	0.030	0.031	....	0	0	....	0.029
12-Year-Old Tree														
Number of leaves	25	876	7,594	21,641	30,216	21,201	8,175	2,343	512	125	0	0	92,708	....
Mean weight per leaf (gm)	0.014	0.060	0.110	0.220	0.370	0.530	0.760	0.950	1.300	1.660	0	0	37.38 kg	....
Mean area per leaf (cm²)	0.06	2.34	4.80	8.73	14.17	20.72	29.08	39.56	53.52	66.47	0	0	146.09 m²	....
Weight per cm² of area (gm)	0.023	0.026	0.023	0.025	0.026	0.026	0.026	0.024	0.024	0.025	0	0	....	0.025
29-Year-Old Tree														
Number of leaves	77	5,444	37,532	55,480	41,839	22,763	7,630	1,624	197	27	0	0	172,613	....
Mean weight per leaf (gm)	....	0.057	0.127	0.224	0.384	0.642	0.885	1.30	....	....	0	0	57.05 kg	....
Mean area per leaf (cm²)	....	2.12	4.68	8.38	13.23	22.03	31.80	48.50	....	....	0	0	202.86 m²	....
Weight per cm² of area (gm)	....	0.027	0.027	0.027	0.029	0.029	0.028	0.027	....	....	0	0	....	0.028

Source: After Turrell (1961).

days showed a sudden and extensive defoliation once water was again applied to the soil (Wedding and Erickson, 1960). The leaves that fell were mature but not senescent. Prior to water application, the leaves wilted and rolled up lengthwise. After receiving water, they regained turgor and their normal shape before dropping.

When citrus leaves age normally, certain specific changes take place in appearance, structure, and composition before they drop. The leaf first turns yellow in the blade and then in the petiole. Starch accumulates temporarily in the mature leaf. Then as the leaf becomes senescent, there is a disappearance of starch from the cortex of the midrib, the starch sheath, the central pith, and to some extent from the blade.

Kelley and Cummins (1920) found that as citrus leaves mature and age the calcium content increases greatly, both in percentage and in amount per leaf. An anatomical study by Scott *et al.* (1948) showed that accumulation of calcium is accounted for largely in the form of calcium oxalate crystals.

The physical process of leaf abscission in citrus appears to begin with a rapid extension of cell walls in some of the parenchyma in the middle cortex of the abscission zone (see chap. 1, fig. 1–13, *A*). The expanding cells create tension between old suberized cell walls and new cellulose, resulting in a rupture of the middle lamella (Scott *et al.*, 1948). After abscission, the enlarging cells round off and present a papillate surface.

Although most changes that precede leaf drop require many days, the extension process may be completed in a few hours for some of the abscission parenchyma. Thus, where leaves literally fall overnight, the necessary stimulus for cell-wall extension must have occurred previously. Water availability is seemingly the only remaining factor required for the rapid cell expansion which separates the leaf. Although Livingston (1950) found that dipping explants of petioles of Valencia orange leaves in water delayed abscission, it is not known whether dipping wilted leaves will elicit the same response. The actual stimulus for abscission may occur as a result of a diminishing auxin gradient across the abscission zone (Addicott and Lynch, 1955).

Stomata and Transpiration

Ambient environmental conditions may have a rapid effect on stomatal guard cells. Light probably exerts a greater influence on stomata than any other environmental factor. Oppenheim (1927) studied daily aperture changes of stomata in sour oranges in Israel. He found that stomata began opening early in the morning, attained a maximum aperture soon after 10 A.M., and remained open completely until after 4:20 P.M. Even though air temperature remained unchanged, stomata closed if clouds temporarily reduced light during the day. Stomatal closure was most obvious between sunset and twilight.

The size of stomata in *Citrus* leaves varies among species. Average stomatal pore lengths and widths determined by Turrell (1947) were: Eureka lemon, 7.04 by 3.08 μ; Marsh grapefruit, 8.06 by 2.08 μ; Valencia orange, 8.91 by 3.80 μ; and Washington navel orange, 4.78 by 2.32 μ. Sometimes the passage of water through the stomata of citrus leaves is retarded by a material that acts as a plug. This material appears in the stomata of both young and old leaves, and its source is still unknown, although it is assumed to be a resin. Plugs of this material are readily seen when fresh leaf sections are studied in water or glycerine. They are not apparent in sections treated with alcohol or xylol. Enough stomata remain functional to regulate transpiration satisfactorily even when some stomata become obstructed (Oppenheim, 1927; Bartholomew, 1931).

Bartholomew (1931) measured transpiration of intact orange and lemon leaves by appressing glass containers with anhydrous calcium chloride against the leaves and then determining weight increases in calcum chloride due to water absorption. As shown in table 2–3, the rate of transpiration from the abaxial (lower) side of leaves was several times greater than from the adaxial (upper) side. In artificial darkness, the transpiration rate decreased by a much greater percentage from the lower side than from the upper, indicating that stomata were functional and responding to changes in light. When transpiration was measured in reverse order, first in darkness and then in light, it was found that the time of day (temperature effects) was not the primary factor altering transpiration. The upper side of citrus leaves are virtually without stomata, and transpiration is thus largely cuticular. The difference between transpiration in light and in darkness is therefore much less than for the lower side with its numerous stomata. Even leaves at least twenty months old were comparatively efficient in regulating their water loss by a functioning of guard cells.

Stomata vary in density on leaves of trees

Table 2–3

STOMATAL RESPONSE OF EUREKA LEMON LEAVES AS INDICATED BY THEIR COMPARATIVE RATES OF WATER LOSS WHEN IN DARKNESS AND WHEN IN BRIGHT, INDIRECT SUNLIGHT[*]

		Rate of Water Loss (Mgm/100 Cm²/Hr)		Average Weather Conditions for Each Test Period		
Test Condition	Test Period	Adaxial Side of Leaves	Abaxial Side of Leaves	Temperature (°F)	Relative Humidity (Per Cent)	Wind Velocity (Mph)
Light	Oct. 15, 10–12 A.M.	86	752	65	49	2.5
Darkness	Oct. 15, 12–2 P.M.	52	227	70	43	4.0
Darkness	Oct. 16, 10–12 A.M.	43	252	70	54	3.0
Light	Oct. 16, 12–2 P.M.	69	684	78	37	4.5

Source: After Bartholomew (1931).

[*] The leaves were approximately fifteen months old, and the same leaves were used in all four tests.

in different geographical areas. In California, Bahgat (1923) demonstrated that certain species and varieties of *Citrus* had a greater density of stomata in hot, arid localities (table 2–4). Density of stomata on the leaves of many species of *Citrus* and related genera growing at Riverside, California, were examined in 1929 by Hirano (1931). Hirano's survey showed that species with a stomatal density lower than 500 stomata per mm^2 came from regions lying between 23.5° and 44° latitude north or south of the Equator, with the exception of *C. aurantifolia* (Christm.) Swing. and *C. cambrovioxora* (possibly *C. macroptera* Montr.). Except for lemon and grapefruit, leaves with a density of more than 500 stomata per mm^2 were found on trees native to the tropics.

Monselise (1951*c*) found that sun leaves of a Shamouti orange tree had 531 ± 9.6 stomata per mm^2 as compared with 412 ± 11.5 stomata for shade leaves. These data indicated that sun leaves had more stomata per unit area and were smaller because of a failure to expand as fully as shade leaves. If sun leaves had expanded to the same extent as shade leaves, the density of stomata would have decreased inversely as the ratio of the leaf sizes or to 415 per mm^2, which is very near the value of 412 per mm^2 found in shade leaves.

In trees subjected to a long summer drought, Mendel (1951) found a low morning peak of both transpiration and stomatal aperture, followed by a decrease that continued for the rest of the day. Trees with rough lemon rootstocks showed remarkable drought resistance, as was reported earlier by Evans (1922). The deep root system of rough lemon probably plays an important part in the drought resistance displayed by this stock.

Unirrigated trees on rough lemon rootstocks had the highest transpiration rates and largest stomatal apertures. Trees on sweet lime rootstocks had intermediate transpiration rates, and trees on sour orange rootstocks had the low-

Table 2–4

ABUNDANCE OF STOMATA ON LEAVES OF SEVERAL CITRUS SPECIES FROM FOUR LOCALITIES IN CALIFORNIA

	Stomata per Square Millimeter on Leaves from Four Localities			
Species	El Centro	Riverside	Porterville	Berkeley
C. paradisi (grapefruit)	617	479	463	499
C. limon (Eureka lemon)	555	530	494	480
C. sinensis (Valencia orange)	465	465	372	......
C. sinensis (navel orange)	550	467	348	358
C. reticulata (mandarin)	......	420	382	363
C. reticulata (tangerine)	......	466	390	376

Source: After Bartholomew and Reed (1943): abridged from Bahgat (1923); adapted from Table II, Hirano (1931).

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est transpiration rates, although the latter two unirrigated rootstocks did not show marked differences in stomatal apertures (Mendel, 1951).

Halevy (1956*a*, 1956*b*) compared the transpiration of attached and detached leaves of Shamouti orange on normal summer days with that on hot, dry days and found that because of stomatal closure the rates of water loss per unit of leaf area were not greatly different. On normal days, the attached leaves transpired 25 to 35 per cent less water than detached leaves. On hot, dry days, the transpiration was as much as 50 per cent lower. On very dry days, stomata were closed when the leaves were picked for weighing. Within two to four minutes, the stomata opened and the transpiration rates of leaves increased markedly over what they were during the first minute after picking (fig. 2–2).

The rate of transpiration varies with the age of the leaf. Small, immature leaves have the greatest rate of transpiration, while young, full-sized leaves lose less water than mature leaves. Old leaves have the lowest water loss. Reed and Bartholomew (1930) believed that the lower rate of water loss of young, light-colored leaves was a factor in their suffering less damage from desiccating winds than older, mature leaves.

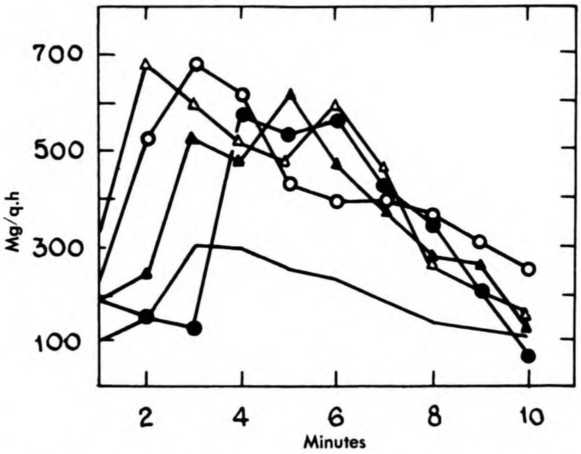

Fig. 2–2. Transpiration of detached leaves in the first ten minutes of a hot, dry day. (Halevy, 1965*a*.)

Photosynthesis and Respiration

Orange and lemon leaves in the expanding stage of development have rates of oxygen uptake several fold in excess of rates of oxygen evolution from photosynthesis (Rhoads and Wedding, 1953), as shown in figures 2–3 and 2–4. In almost mature leaves that have not hardened, the photosynthesis rate may be as high as the respira-

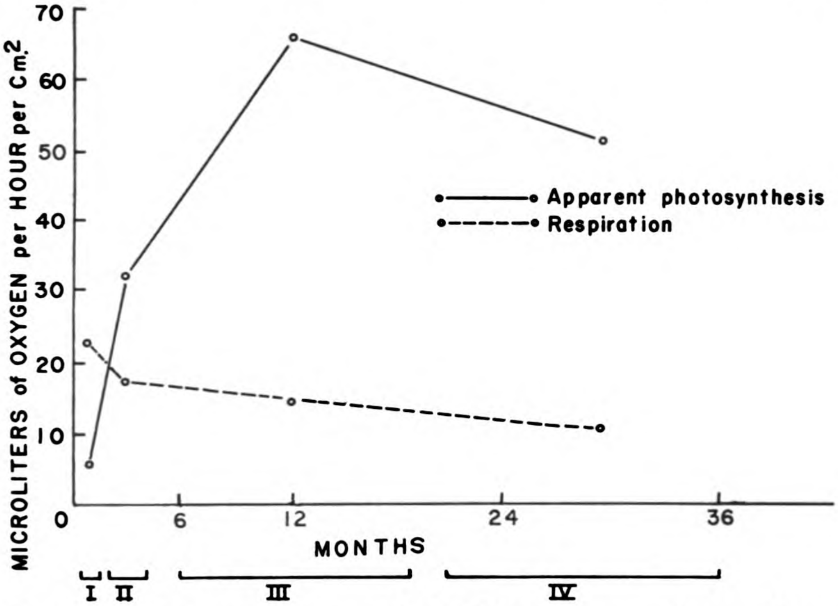

Fig. 2–3. Rates of respiration and photosynthesis of Washington navel orange leaves. Group I, very small leaves, characteristically found at tips of new growth flushes. These were sampled when they were approximately 2 cm wide and 3 to 4 cm long. Group II, young leaves found in the new growth flushes which had almost reached maturity but not hardened. Group III, mature leaves which had not begun to show signs of aging. Group IV, old leaves that were not showing obvious signs of aging. (Rhoads and Wedding, 1953.)

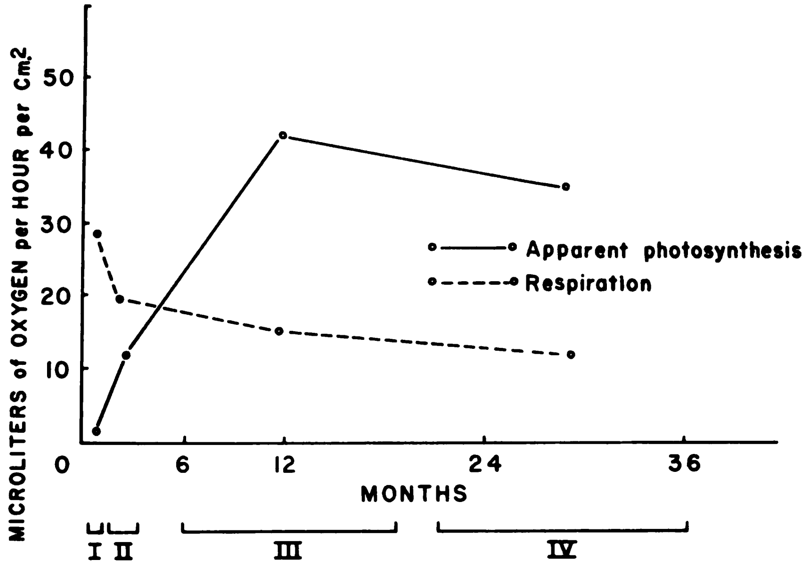

Fig. 2–4. Rates of respiration and photosynthesis of Eureka lemon leaves. Group I, very small leaves, characteristically found at tips of new growth flushes. These were sampled when they were approximately 2 cm wide and 3 to 4 cm long. Group II, young leaves found in the new growth flushes which had almost reached maturity but not hardened. Group III, mature leaves which had not begun to show signs of aging. Group IV, old leaves that showed no obvious signs of aging. (Rhoads and Wedding, 1953.)

tion rate. The respiration rate decreases in leaves throughout their lives, whereas the rate of photosynthesis increases to a maximum within about a year, only to decrease as the leaves age further.

Monselise (1951*b*) grew sweet lime seedlings from May until October in full sunlight and under reduced illumination ranging from 13 to 30 per cent of full sunlight. Seedlings under reduced radiation grew better and became larger than sun seedlings. A comparison of the photosynthetic activity of trifoliate orange and sweet lime leaves showed that the former were more active on both area and weight bases.

Monselise (1951*c*) found that only the very exterior leaves of citrus trees were exposed to high light intensity. In the interior of the tops of citrus trees, light intensity was mostly 2 per cent or less of full sunlight. Clementine mandarin and Marsh grapefruit trees were denser than Shamouti orange. Although citrus trees have a high shade tolerance, maximum yields are produced only under high light intensity. Monselise (1951*a*) found that light conditions and yields were improved when overcrowded groves were thinned.

Leaf Composition

Calcium oxalate occurs abundantly as rhomboidal crystals in large cells in the palisade layers, in endodermal cells near vascular bundles, and in tissue near the lower epidermis of leaves. Often hesperidin is present, especially in young leaves. When tissues are dried, frozen, or treated with alcohol, hesperidin may be precipitated as needle crystals.

Subepidermal oil glands in the leaves of *Citrus* appear to arise lysigenously through the dissolution and disorganization of cells. Mechanical injury or abnormal pressures from surrounding tissues may cause the glands to release oil, but otherwise they remain closed. Oil glands are particularly noticeable in the leaves of certain species such as lemon and citron. No evidence exists that oil is a reserve food.

The essential leaf oils of *Citrus* are primarily hydrocarbons, mostly terpenes and sesquiterpenes. Oil composition varies greatly between different species and varieties. Oil from lemon leaves, for example, contains several times as much citral of both isomers (geranial and neral) as oil of orange leaves (Finnemore, 1926; Scora and Torrisi, 1966). Age also has been found to greatly influence the composition of essential oil in leaves. Scora and Torrisi (1966) found that the amount of citral in two-month-old Valencia leaves was appreciably higher than in nine- to ten-month-old leaves.

Starch is a common constituent of citrus leaves, ranging in concentration from a trace to as high as 5 or 6 per cent in dry matter, depending upon climatic, seasonal, varietal, and other factors. Starch in the mature citrus leaf is slower to disappear than in the leaves of many other plants. The chloroplast apparently binds the natural starch of the leaf, and it is digested very slowly by the leaf enzyme (Sinclair and Bartholomew, 1937). Mature leaves of potted orange seedlings were found by Bartholomew to be high in starch even after five weeks in a dark room.[1] When macerated leaf tissue reacted on a starch solution at a pH between 4 and 5.4, conversion of starch to sugars occurred rapidly, indicating that the enzyme itself was active. Mature leaves were shown to have far less diastatic activity than young leaves. Temperature changes effected similar responses in diastatic activity of both young and old leaves. Citrus leaves achieved maximum diastatic activity at temperatures ranging from 60°C to 65° C.

Under the climatic conditions of Arizona, Martin (1940) studied young grapefruit leaves produced in the spring and found starch to be almost absent until the next December, when it increased rapidly. Sharples and Burkhart (1954), also working in Arizona, showed that starch decreased in the leaves of grapefruit trees during the growing season, while soluble carbohydrates increased, particularly in December. During January and February, the starch content increased rapidly in one-year-old leaves and the total soluble carbohydrates also showed a comparable decrease. A marked conversion of starch to sugars occurred during the onset of spring growth.

In leaves of Valencia orange and Eureka lemon in California, sugar and starch are at a minimum during the summer months. Sugar is at a maximum during the winter months, whereas starch remains low. The sugar increase during the winter months, without an accompanying starch increase, probably constitutes a "cold reaction" in citrus. There is a very rapid starch increase early in the spring, just before the spring flush of growth. During the spring growth period, the starch buildup disappears. Other flushes of growth also may be preceded by an accumulation of starch in the leaves (Jones and Steinacker, 1951). The total accumulation of carbohydrate materials in the leaves may be influenced by the fruit load on the tree (Jones *et al.*, 1964). In the Florida climate, there is only a slight accumulation of starch in Valencia orange leaves during winter which is soon exhausted after the advent of spring growth (Smith, Reuther, and Specht, 1952).

Research by Sinclair and Bartholomew (1937) showed that concentrations of $ZnSO_4$ and $ZnCl_2$ applied at rates of up to 30 meq/l did not inhibit the diastase activity in Valencia orange leaves. Zinc treatments of citrus trees are designed to cure or prevent mottle-leaf.

Pretreatment of citrus seeds with a mercury compound has been shown to affect the seed enzyme system in such a way that healthy instead of albinistic seedlings are produced (Perlberger and Reichert, 1938).

Reed (1921) found that sap expressed from mature orange leaves has an osmotic pressure of about 18 atmospheres (measured by the freezing-point depression). He reported little fluctuation during the growing season. In contrast, expressed sap of young leaves is much less concentrated.

The amount of dry matter in citrus leaves ranges from about 29 per cent in full-sized young leaves to 45 per cent in mature leaves twelve- to eighteen-months-old, depending upon leaf age and various other conditions. Dry matter of citrus leaves from various combinations of scion and rootstock was shown by Haas (1937) to contain 11.92 to 18.43 per cent of pectinaceous material expressed as a percentage of calcium pectate. Prior to and during new growth and blooming, the nitrogen content of mature grapefruit leaves has been found to undergo a reduction (Hilgeman, Smith, and Draper, 1940). In some instances, Hilgeman *et al.* found it possible to detect a nitrogen content increase in grapefruit leaves within fifteen days after applying nitrogen to the soil. Martin (1940) reported that the accumulation of carbohydrates in the leaves and twigs of grapefruit was reduced by a deficiency of nitrogen.

The amino acids commonly found in citrus leaves (Stewart, 1962) are alanine, γ-aminobutyric acid, asparagine, aspartic acid, glutamic acid, lysine, proline, serine, and threonine. Others usually present are glutamine and glycine. Amino acids found in lesser amounts are histidine, the leucines, phenylalanine, pipecolic acid, tryptophane, tyrosine, and valine. Cysteine, cystine, and ornithine have not been identified with certainty. Proline is present in citrus leaves and fruit in greater quantity than any other amino acid.

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[1] Unpublished data from a study (Project 824) made in 1934 by E. T. Bartholomew and on file at the University of California Citrus Research Center and Agricultural Experiment Station, Riverside.

Minor element nutrition greatly influences the free amino acids in citrus leaves. Each deficiency has its characteristic effect. Stewart (1962) found, as shown in table 2–5, that chlorotic leaves from iron-deficient trees had more arginine, asparagine, aspartic acid, serine, and threonine, and less alanine than leaves from control trees, but green leaves from deficient trees contained less alanine and threonine than controls.

Zinc-deficient trees showed an accumulation of arginine and proline, but contained less alanine than the controls. Manganese-deficient leaves had more aspartic acid and proline, but less γ-aminobutyric acid than normal leaves. Magnesium-deficient leaves had more tryptophane and serine, but less alanine than controls.

Although young leaves on copper-deficient plants showed no differences in amino acids from controls, the older deficient leaves had more aspartic acid, glutamic acid, proline, serine, and threonine.

Since the various minor elements are to be found in the metal-containing proteins and enzymes, a deficiency of one or more of these elements may be expected to limit the synthesis of the proteins of which they are a part. Thus, the accumulation of certain amino acids in response to a minor-element deficiency might be an indication of the relative abundance of these amino acids in the protein whose synthesis has been limited.

Leaves abscising at an age of nine or ten months returned essentially no nitrogen to the tree before abscission, whereas older leaves returned as much as 56 per cent of their nitrogen (Wallace *et al.*, 1954). In a comparison of green leaves with ones about to abscise, it was found that the major difference in nitrogen content was in the water-insoluble fraction.

In experiments with N^{15}, it was found that nitrogenous compounds are constantly being hydrolyzed and resynthesized in citrus, as in other species of plants.

Samples of macerated leaf tissue of Lisbon and Eureka lemons and Washington navel and Valencia oranges were found to exhibit urease activity (Kuykendall and Wallace, 1954). The amount of urease activity in intact leaves was similar to that in extracts. The activity of extractable urease from young leaves was about twice as great as that in mature leaf tissue.

Detached citrus leaves readily absorbed urea from 2 and 4 per cent solutions in which they were submerged. Valencia orange leaves absorbed urea at a faster rate than Eureka lemon leaves. Three-month-old Eureka lemon leaves absorbed urea about three times as fast as mature leaves. The rate of absorption was roughly proportional to the concentration of urea in the solution. High temperatures favored more rapid absorption than low temperatures in the range from 4°C to 37°C. Initial absorption (Impey and Jones, 1960*a*) from the upper leaf surface was much less than from the lower leaf surface, but after 30 hours total absorption was approximately equal.

Biuret, a toxic impurity in urea fertilizers, produces yellow or chlorotic leaf tips in citrus (Jones, 1954). The injured leaf tips characteristically have a higher water-soluble nitrogen fraction and a lower chlorophyll and lipid-nitrogen fraction than normal leaves (Impey and Jones, 1960*b*). The increase in water-soluble nitrogen in injured leaves was found to be chiefly in increased amounts of serine, glycine, glutamine, lysine, arginine, ornithine, and four unknown amino acids. The authors suggested that the increase in glycine and decrease in chlorophyll were related and that the biuret could be involved in either the synthesis or destruction of chlorophyll. Biuret was detected in the injured leaf tips eight months after application, indicating that citrus leaves have no mechanism for utilizing biuret.

Kessler and Monselise (1961) found that ribonucleic acid (RNA) content and ribonuclease activity rise rapidly in orange leaves during the first ten weeks of their development (figs. 2–5 and 2–6). In twenty-six-week-old orange leaves, the ribonucleic acid content was found to be somewhat higher than in ten-week-old leaves, whereas the ribonuclease activity was about the same in leaves of these two ages. This situation was similar in leaves from one-, four-, and twenty-five-year-old trees, although the levels were different. Progressively older trees were found to have progressively higher nucleic acid contents in comparable leaves of all ages. By contrast, the progressively older trees were found to have progressively less ribonuclease activity in older, comparable leaves. The rapid rise in ribonuclease activity in developing leaves rendered the relationship with tree age somewhat uncertain.

Table 2–5

FREE AMINO ACIDS IN FLORIDA ORANGE LEAVES FROM HEALTHY TREES AND THOSE HAVING MINOR ELEMENT DEFICIENCIES

Amino Acids	– Fe Chlorotic Leaves	– Fe Green Leaves	+ Fe	– Zn Chlorotic Leaves	– Zn Green Leaves	+ Zn	– Mn	+ Mn	– Mg	+ Mg	Old Leaves		New Leaves	
											– Cu	+ Cu	– Cu	+ Cu
				Expressed as Micrograms of Amino Nitrogen per Gram of Dry Weight†										
Aspartic acid	23**	13	12	17	7*	14	23*	10	34	28	18**	7	15	13
Glutamic acid	7	7	8	15	7	6	9	9	8**	13	12	6	24	24
Serine	277**	85	87	83*	61	71	74	78	131**	68	105**	41	247	232
Threonine	32*	15**	19	23**	24**	12	27*	20	25**	15	20**	8	18	17
Alanine	24*	21*	35	35**	46**	75	49*	61	49**	78	84*	59	139	158
γ-Aminobutyric acid	129	114	270	107**	181	168	135**	232	114**	135	259**	141	355	260
Proline	1,467	1,180	1,598	2,006**	1,533**	845	1,106	762	2,848**	1,118	1,002**	350	767	825
Asparagine	11*	4	2	T	T	T	T	T	T	T	T	T	34	25
Tryptophane	....	....	....	T	T	T	T	T	43**	T	T	T	T	T
Arginine	170**	7	11	1,358**	28**	11	10	12	26	19	12	9	29	31
Lysine	8	4	6	....	....	....	....	....	....	....	....	....	....	....
Total amino nitrogen‡	2,148	1,450	2,048	3,644	1,887	1,202	1,433	1,184	3,278	1,474	1,512	621	1,628	1,585
					Expressed as Per Cent Dry Weight									
Total nitrogen	2.18	2.17	2.40	2.50**	2.76	2.65	2.78	2.65	2.40*	2.62	2.66**	1.99	4.09	4.06

Source: Stewart (1962).

* Indicates that the mean differs significantly from control mean by odds greater than 19 to 1.

** Indicates that the mean differs significantly from control mean by odds greater than 99 to 1.

† T — trace, < 1.0 μgm of amino N/gm.

‡ Statistical significance not determined.

Table 2–6

RNA, DNA, AND TOTAL NITROGEN PER AVERAGE LEAF IN DEVELOPING SHAMOUTI ORANGE LEAVES IN ISRAEL

Approximate Age of Leaves (Weeks)	RNA (μgm)	DNA (μgm)	RNA/DNA	Total N (Mgm)
4	867	217	4.0	4.47
6	1,249	265	4.7	5.35
10	2,280	357	6.4	9.25
26	3,524	319	11.0	15.28
SE†	285‡	32‡	0.45	1.48‡
F Value	17.6*	1.86	48.4*	11.4*

Source: After Monselise, Cohen, and Kessler (1962).

* Significant at 0.01 level.

† Standard error.

‡ The statistical treatment does not include the sample taken at 4 weeks.

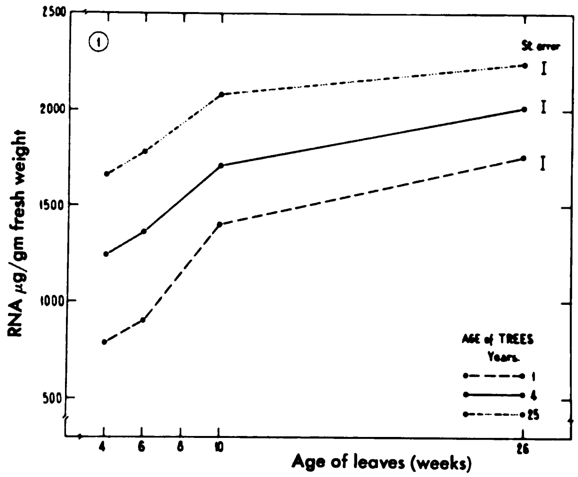

Fig. 2–5. Ribonucleic acid content of developing Shamouti orange leaves as influenced by leaf and tree age. (Kessler and Monselise, 1961.)

In contrast to the rapid rise of RNA in developing citrus leaves, deoxyribonucleic acid (DNA) remains quite constant (table 2–6). Application of purine and pyrimidine bases were found to influence the amounts of RNA and DNA. Uracil, xanthine, and caffeine enhanced RNA synthesis, whereas adenine promoted DNA accumulation (Monselise, Cohen, and Kessler, 1962).

Chlorophyll synthesis is also influenced by these materials. Kessler, Cohen, and Monselise (1960) found that adenine stimulates chlorophyll formation in leaves shortly after application. However, uracil, xanthine, and caffeine tended to result in an initial decrease in chlorophyll, although later in the season there was some stimulation of chlorophyll formation.

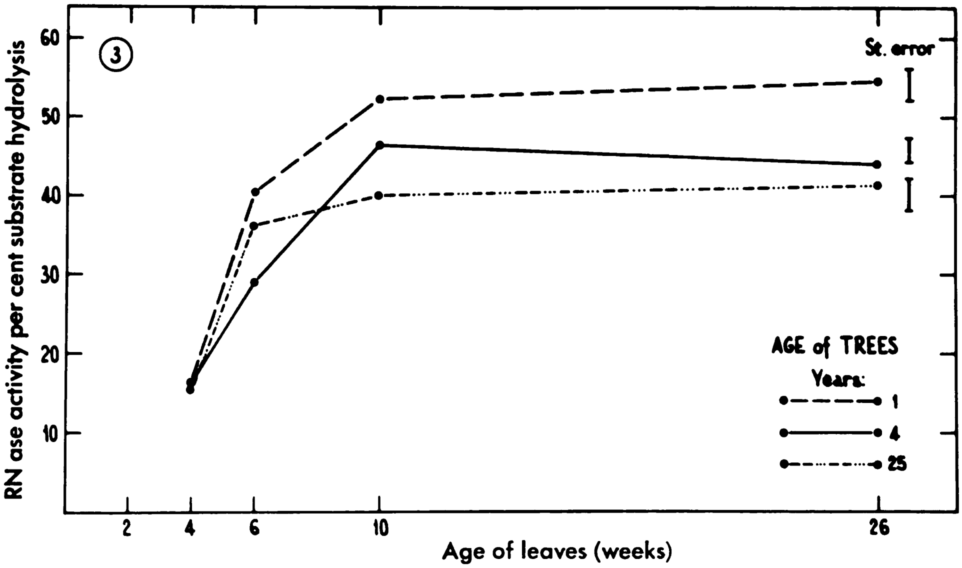

Fig. 2–6. Ribonuclease activity of developing Shamouti orange leaves as influenced by leaf and tree age. (Kessler and Monselise, 1961.)

FRUIT DEVELOPMENT AND PHYSIOLOGY

Flowering and Setting

Although seasonal conditions may cause citrus to bloom at various times, the main blooming period of citrus trees in subtropical climates is in the spring. Ayalon and Monselise (1960) found that Shamouti orange-flower induction stimuli were largely operative in the early part of January, whereas microscopic evidence of differentiation was not evident until late in January. Various factors such as disease and rainfall or heavy irrigation preceded by a dry period may promote blooming of a branch, a whole tree, or even an orchard at any time of year. Fruit from an irregular bloom—called an off-bloom— is usually inferior. In the coastal and intermediate valleys of southern California, the Eureka-type lemon tends to bloom throughout the year, more heavily in the spring than at other times. In hot, tropical climates near the Equator, all citrus tends to bloom throughout the year, except as influenced by periods of drought.

Although citrus trees usually bloom heavily, a comparatively small percentage of flowers produce mature fruit, since many flower buds and flowers drop before fruit is set. Many fruits that set drop before reaching maturity (table 2–7).

In a study of the relationships between flowers and fruits of the lemon, Reed (1919) determined that out of 4,440 lemon flower buds 52 per cent set fruit, 21 per cent of these fruits attained a diameter of 0.25 inch, and only 7 per cent reached full maturity.

Shavit (1956) found that 77 per cent of Shamouti orange flowers examined were defective and another 6.2 per cent were staminate. In normal trees that were not zinc-deficient, 15.6 per cent of flowers were shed before they opened,

Table 2–7

AVERAGE NUMBER OF REPRODUCTIVE STRUCTURES (BUDS, FLOWERS, AND FRUIT) ABSCISED PER TREE FROM WASHINGTON NAVEL AND VALENCIA ORANGE TREES AT RIVERSIDE, CALIFORNIA, 1958–59

	Washington Navel Orange					Valencia Orange				
Diameter of Ovary (Mm)	Buds	Flowers	Fruit With Pedicel	Fruit Without Pedicel	Total	Buds	Flowers	Fruit With Pedicel	Fruit Without Pedicel	Total
1 (or less)	59,635	674	1,002	12	61,323	20,020	341	893	173	21,427
2	22,790	14,939	13,953	344	52,026	4,099	565	5,468	2,621	12,753
3	10,804	15,295	25,043	541	51,683	1,365	1,031	11,151	1,880	15,427
4	3,114	2,321	13,469	589	19,493	499	219	7,610	2,533	10,861
5	....	6	3,853	460	4,319	....	7	1,956	2,161	4,124
6	....	....	2,399	634	3,033	....	....	702	1,898	2,600
7	....	....	1,250	643	1,893	....	....	137	1,164	1,301
8	....	....	586	450	1,036	....	....	59	582	641
9	....	....	417	663	1,080	....	....	21	699	720
10	....	....	179	462	641	....	....	5	611	616
11	....	....	77	357	434	....	....	2	453	455
12	....	....	37	275	312	....	....	1	392	393
13	....	....	20	194	214	....	....	5	297	302
14	....	....	7	137	144	....	....	....	237	237
15	....	....	7	118	125	....	....	....	158	158
16	....	....	3	79	82	....	....	....	106	106
17	....	....	2	71	73	....	....	....	83	83
18	....	....	2	55	57	....	....	....	65	65
19	....	....	2	40	42	....	....	....	34	34
20	....	....	....	32	32	....	....	....	23	23
21 (or more)	....	....	....	232	232	....	....	....	61	61
Total	96,343	33,235	62,308	6,388	198,274	25,983	2,163	28,010	16,231	72,387
Mature fruits per tree					419	Mature fruits per tree				708
Total flower buds per tree					198,693	Total flower buds per tree				73,095
Per cent	48.5	16.7	31.4	3.2	crop = 0.2	35.5	3.0	38.3	22.2	crop=1.0

Source: Erickson and Brannaman (1960).

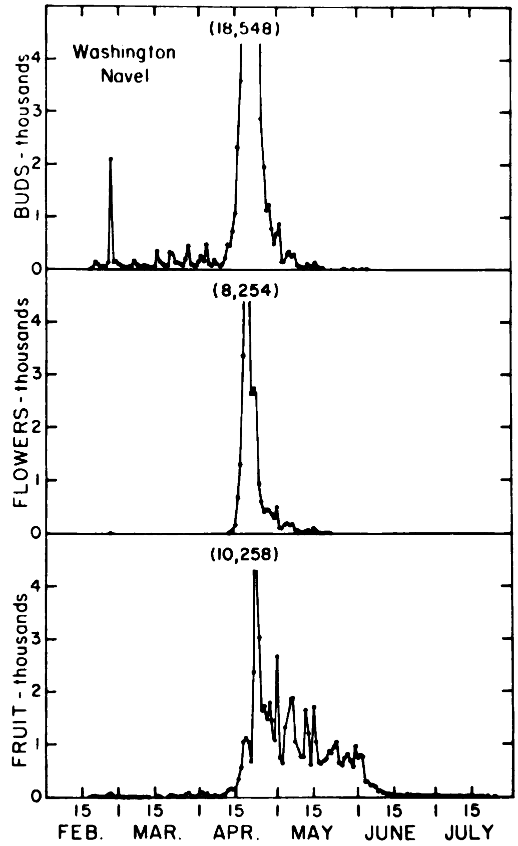

Fig. 2–7. Average daily drop of flower buds, flowers, and fruits from five Washington navel orange trees, 1958. (Erickson and Brannaman, 1960.)

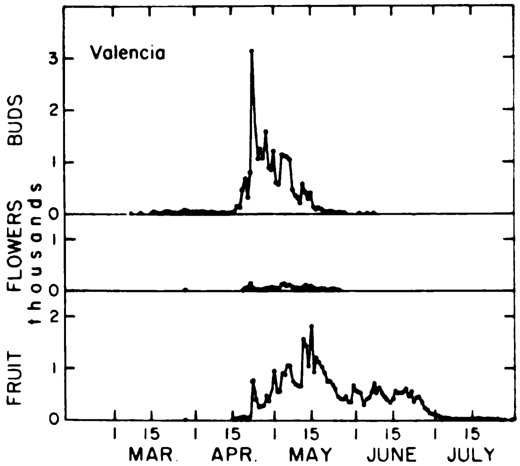

Fig. 2–8. Average daily drop of flower buds, flowers, and fruit from five Valencia orange trees, 1958. (Erickson and Brannaman, 1960.)

whereas 18.8 per cent shed in the stage of well-developed, open flowers. The stage of young fruit was reached by 45.1 per cent of the total flowers. In contrast, zinc-deficient trees shed 35.8 per cent of flowers in the bud stage and 13.4 per cent in the open stage. Also, zinc-deficient trees were found to have 11 per cent staminate flowers, and only 23.8 per cent of the flower buds developed to the young fruit stage.

The flowers on individual Shamouti orange trees that produced mature fruit varied from between 2.23 and 5.33 per cent. Erickson and Brannaman (1960) recorded similar natural thinning in Valencia oranges and Washington navels; the former variety matured 1.0 per cent of the potential crop and the latter, 0.2 per cent.

The primary cause controlling the dropping of flowers and young fruit is a weakening of tissue in a preformed abscission zone at the point of attachment of the base of the ovary to the disk or of the pedicel to the twig. The actual mechanism of the process is not well understood, but it is believed to be triggered by growth regulators in response to either external or internal changes. Young fruits that remain after the first period of dropping presumably are capable of developing to maturity if weather is favorable. Some dropping of fruits occurs throughout the season, however. As the weather becomes hot in the early summer months, there is generally a period of accelerated fruit drop. In the United States, this is referred to as the "June drop" period (Coit and Hodgson, 1919). The drop may be light and not significantly affect the crop (figs. 2–7 and 2–8). Under severe heat prolonged for several days, however, drop is usually heavy, resulting in a greatly reduced crop. Temperatures well over 100°F in June, 1917, and again in June, 1957, resulted in almost complete losses of the Washington navel crops in the San Bernardino and Redlands areas of California (Coit and Hodgson, 1919; Platt, 1958). Hilgeman, Dunlap, and Sharp (1967) also noted that prolonged heat and high temperatures in May reduced the crop of Valencia oranges in Arizona. Har-Even and Monselise (1959) recorded a severe drop of Shamouti oranges at Rehovat, Israel, in May and June, 1957, after a hot, dry, windy period near the end of May.

In a study of factors affecting yield of Washington navel oranges at Riverside, California, from 1926 to 1963, Jones and Cree (1964) reported a negative correlation coefficient of 0.510

between the single-day maximum temperature for the period of May 10 to June 30 and yield. This covers the June drop period. Evidently several days of high temperatures are required to produce an abnormal drop.

With fruits falling during June, the abscission layer usually forms between the base of the ovary and the disk. This is also the case with ripe fruits that fall.

Fruit Growth

Waynick (1927) measured the growth in diameter of Valencia oranges from September 1 until the following summer. Maximum growth occurred between September 1 and about December 1, after which the rate of increase in fruit diameter fell off. Waynick found that fruit size on December 1 was proportional to fruit size at picking time and therefore useful in forecasting fruit sizes. The growth of the interior fruit on the tree was slower than that of outside fruit during the period up to December, but the continued enlargement of the inside fruit resulted in approximately equal sizes at maturity (fig. 2–9).

Bain (1958) found oranges tended to accumulate soluble solids most in the final stages of fruit development when increase in size was only slowly taking place. Possibly the longer period of growth exhibited by interior fruit indicates that the final stage of maturation involving the accumulation of soluble solids is also delayed and perhaps shortened, thus accounting for the lower soluble solids found in inside fruit by Sites and Reitz (1949).

Waynick (1928) noted a correlation between smaller sizes and the high percentage of cloudy days through the spring of 1928. The break in the growth curve of the fruit about December 1 was closely associated with a change of color from green to orange.

Almost complete defoliation of trees in October resulted in a reduction in fruit by two packinghouse sizes (about 6 mm in diameter) at maturity. Dry winds with humidity as low as 4 per cent reduced fruit size irreversibly. Exposed fruits always colored more rapidly, and even in cases where no mechanical injury was shown there was clearly evidence of retarded growth associated with premature coloring of the fruits. Waynick (1927) attributed premature coloring to extreme desiccation of the outer cells of the fruit.

Bain (1958) divided Valencia orange fruit development into three stages: Stage I is a period of cell division, which occurs in the southern hemisphere in November and part of December. This period includes flowering and formation of the various tissues in the small fruits. Respiration per unit weight of tissues is high initially in the flowers and then falls off during the first stage of growth (fig. 2–10). Stage II follows the period of cell divi-

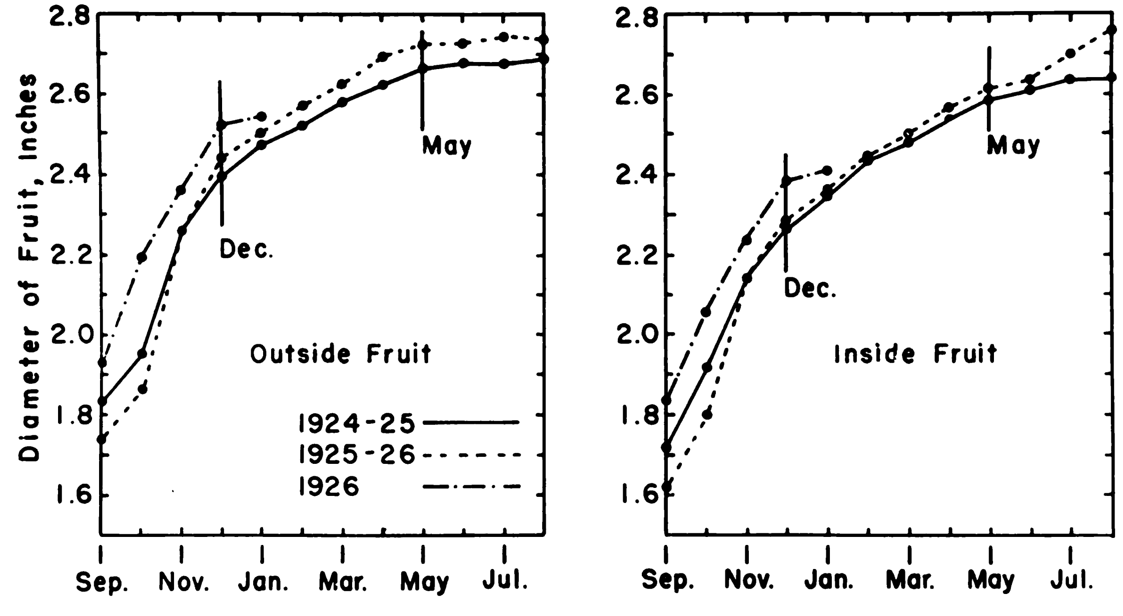

Fig. 2–9. Growth rates of Valencia orange fruits. Left, outside fruits. Right, inside fruits. (Waynick, 1927.)

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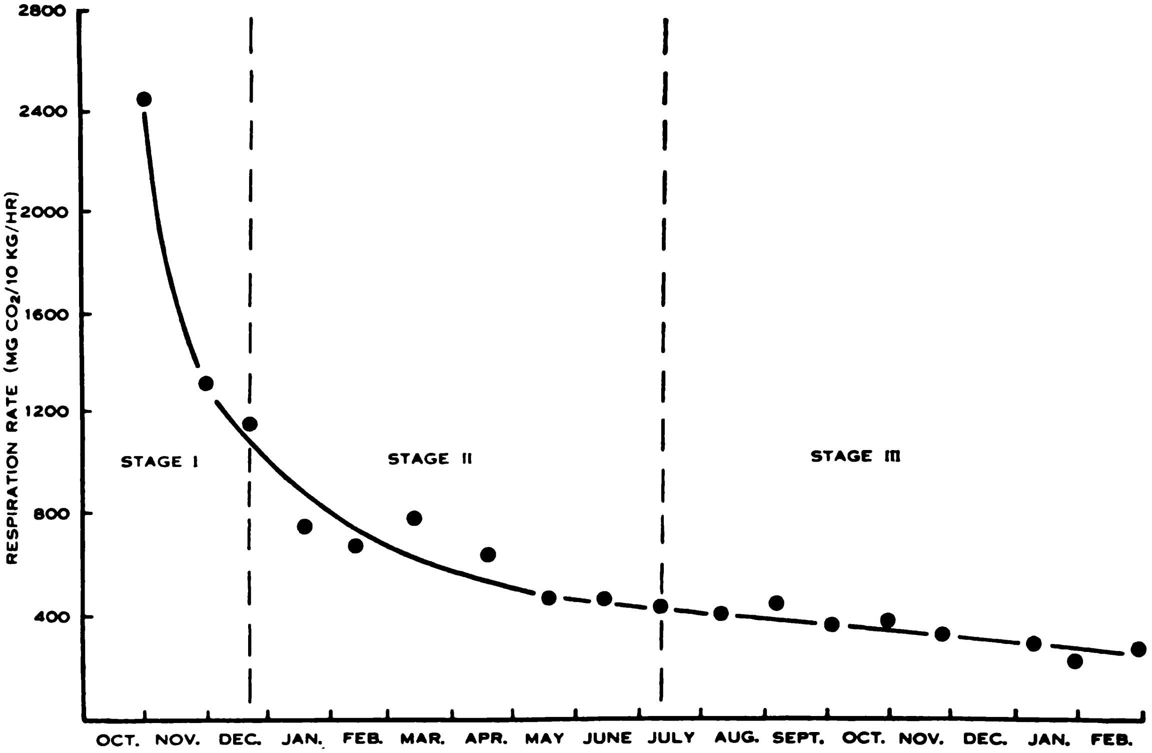


Fig. 2–10. Respiration rates on a fresh-weight basis throughout Valencia fruit development (measured at 20°C). (From Bain, 1958.)

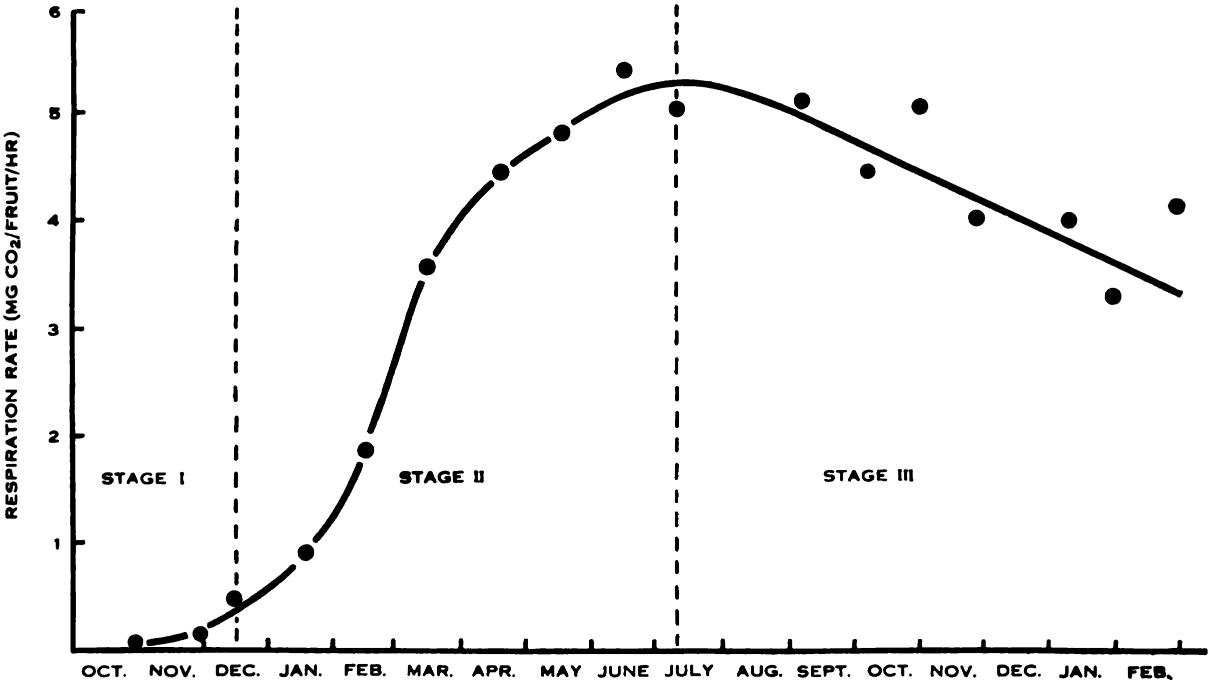


Fig. 2–11. Respiration rate per Valencia fruit throughout development (measured at 20°C). (From Bain, 1958.)

sion and is characterized by rapid enlargement of cells in the fruit. Although respiration per fruit climbs rapidly during this stage of development (fig. 2–11), the rate per unit weight of tissue continues to decline. During the latter part of Stage II, peel changes in color from green to orange. Stage III is regarded as the maturation period. It is characterized by a reduced rate of enlargement and compositional changes associated with maturation. Changes in constituents consist mainly of increases in absolute amounts of soluble solids in the form of sugars and nitrogenous compounds to keep pace with additional fruit enlargement. Citric acid, however, continues to decrease in concentration, a change which began during the second stage of fruit development.

Fruit Composition

Amino Acids.—The free amino acids constitute an important fraction of the soluble solids in citrus juice. Aside from their buffering effects in the cells, which might be performed by other organic acids, they are indispensable structural units of the proteins.

During fruit development, the free amino acid content of the juice varies quantitatively and qualitatively (table 2–8). In early stages of Valencia orange development, asparagine is the most abundant amino acid, followed by serine and aspartic acid (Clements and Leland, 1962). Free ammonia is even more abundant than these amino acids in the immature orange, but decreases to a negligible level as the fruit matures.

Proline increases more than any other amino acid during the maturation of Valencia oranges. In mature fruit, it may account for 2.67 per cent of the solids in the juice (Clements and Leland, 1962). Proline also is most abundant in Washington navel oranges, Dancy tangerines, Eureka lemons, and Lisbon lemons, but second to aspartic acid in concentration in Marsh grapefruit (fig. 2–12).

Eureka and Lisbon lemons differ from other citrus varieties mentioned above, not only in the generally lower concentration of total amino acids in the juice, but also in their relative proportions of these acids.

Thus, asparagine and arginine are relatively low in lemons and the ammonia content is relatively high. Differences in concentration also exist in other amino acids, but not to such a great degree.

Organic Acids.—Citric acid is the characteristic organic acid of citrus fruits. It accounts for most acidity in the pulp of those fruits with a few exceptions, such as the sweet lime, where it is so low that malic acid exceeds it (Clements, 1964*a*).

Citric acid accumulates most rapidly in young, developing fruits. Bartholomew (1923) found, for example, that lemons with a diameter of 2 cm had only 0.26 per cent acid, those with a diameter of 3 cm had increased to an average of 1.05 per cent, those with a diameter of 4 cm averaged 4.26 per cent, those with a diameter of 5 cm averaged 4.92 per cent, and those with a diameter of 6 cm averaged 5.35 per cent. In terms of grams of citric acid per fruit, the accumulation of acid in lemons is readily seen to be more rapid than the diluting effect resulting from increases in fruit size. Although lemons and limes commonly have an acid concentration of about 5 or 6 per cent (Bartholomew and Sinclair, 1951; Erickson and Brannaman, 1950), it may range as high as 9 per cent in lemons (Money and Christian, 1950).

In oranges, on the other hand, although acid accumulation is rapid in young fruits, it decreases in concentration in later stages of development (Sinclair and Ramsey, 1944). Rasmussen (1964) found that the total citric acid per fruit in Valencia oranges declined by at least two-thirds as the fruit became thoroughly mature.

Characteristically, the acid concentration of the juice of grapefruit and oranges decreases steadily during maturation (Harding, Winston, and Fisher, 1940; Sinclair and Bartholomew, 1944; Harding and Fisher, 1945; Rygg and Getty, 1955; Bain, 1958). In California, the acidity of mature oranges usually ranges between 1 and 1.3 per cent (Sinclair, 1961). In Florida, it usually ranges between 0.5 and 1 per cent (Harding, Winston, and Fisher, 1940). Varieties, rootstocks, mineral nutrition, and climatological factors all exert influences on fruit acidity. These factors are discussed more fully in other chapters in this volume and in Volume III.

Mandarins have about the same acidity range as oranges (Kefford, 1959). Grapefruit is commonly more acid. Rygg and Getty (1955) studied the seasonal changes in Arizona and California grapefruit during three seasons. Their data showed a decreasing acidity in the desert fruit from the earliest sampling in October, when average acidity was about 1.8 per cent. By the end of June, average acidity had decreased to about

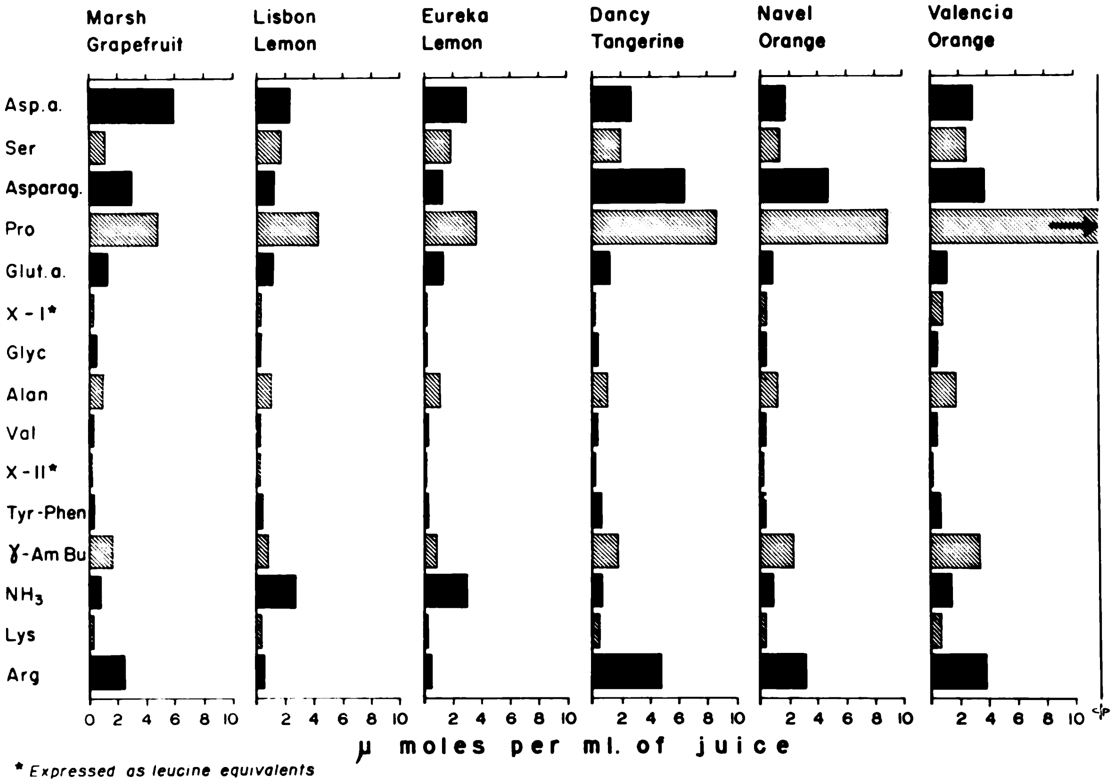
Table 2–8

SEASONAL VARIATIONS IN THE FREE AMINO ACIDS IN VALENCIA ORANGE JUICE AT RIVERSIDE, CALIFORNIA

Amino Acids	Seasonal Variations (μmoles per Ml Juice)													
	Sept. 30, 1959	Nov. 2	Dec. 1	Jan. 15, 1960	Feb. 24	Mar. 16	Apr. 4	May 4	June 1	July 5	Aug. 29	Oct. 5	Nov. 4	Dec. 12
Alanine	0.7	0.6	0.7	1.1	1.1	1.1	1.4	1.3	1.7	1.7	2.2	3.0	4.0	4.5
γ-Aminobutyric acid	0.8	0.9	0.9	1.3	2.0	2.1	3.3	2.4	3.3	4.1	5.6	6.8	9.4	10.0
Ammonia	6.0	3.5	2.6	1.5	1.1	1.0	1.1	0.7	0.8	0.6	0.3	0.5	0.3	0.3
Arginine	0.3	0.6	1.4	1.9	2.2	2.6	3.0	4.2	4.4	5.2	6.0	6.9	9.3	12.0
Asparagine	5.2	3.5	2.8	2.6	3.1	2.5	3.6	2.7	2.3	2.8	3.7	3.4	3.9	4.5
Aspartic acid	2.7	3.5	3.1	2.4	2.6	2.3	2.5	2.3	2.5	3.0	3.5	3.6	4.6	5.3
Glutamic acid	1.3	0.9	1.2	0.9	1.2	0.9	1.1	1.3	1.2	1.2	1.0	0.9	0.9	0.8
Glycine	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.4	0.7	0.8
Lysine	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.6	0.9	0.8
Phenylalanine + tyrosine	0.2	0.2	0.2	0.3	0.2	0.3	0.4	0.5	0.5	0.5	0.5	0.7	0.8	0.8
Proline	1.5	3.5	5.1	9.1	14.2	14.0	19.0	15.0	16.0	17.7	18.5	23.9	34.8	38.2
Serine	3.5	2.0	1.7	1.7	1.8	1.7	2.1	1.8	1.7	1.8	1.9	2.0	2.6	3.2
Unknown VIII*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.3
Unknown III*	Tr.	Tr.	Tr.	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.5	0.8	1.3	1.1
Total μmoles amino acids per ml	22.5	19.5	20.0	23.4	30.1	29.1	38.2	33.1	35.4	39.8	44.7	53.7	73.8	82.6
Total μmoles N per ml (from amino acids)	28.7	24.9	27.1	31.9	40.0	39.6	51.1	48.7	51.3	58.6	66.9	78.4	106.5	123.9
Total μmoles N per ml (Kjeldahl)	46.8	36.6	41.1	46.8	56.2	56.6	70.1	64.6	71.8	77.8	93.8	106.8	136.2	149.0
Mgm N per ml (Kjeldahl)	0.66	0.51	0.58	0.66	0.79	0.79	0.98	0.90	1.01	1.09	1.31	1.50	1.91	2.09
N accounted for (%)	61	61	66	68	71	70	73	75	71	75	71	73	78	83

Source: Clements and Leland (1962).

* Expressed as leucine equivalents.



* *Expressed as leucine equivalents*

Fig. 2–12. The principal amino acids in six varieties of citrus. (Clements and Leland, 1962.)

1 per cent. Evaluation of the fruit for palatability indicated it was acceptable from about the end of October to the end of June. In averaging their data for ten desert orchards in Arizona and California, Rygg and Getty found that at the time of maximum fruit acceptability, as determined by taste tests, average acidity was 1.47 per cent. Other averaged characteristics of this fruit at maximum acceptablility were as follows: 11.06 per cent soluble solids, a soluble solids-to-acid ratio of 7.69, 40 mgm ascorbic acid per 100 ml of juice, and a flavor rating of 87 (minimum acceptable level=70). These values were found to apply on about April 5. Coastal grapefruit usually did not achieve minimum acceptability until July. The authors indicated that a ratio of 6.5 soluble solids-to-acid should be the minimum from both desert and coastal areas for consumer acceptability.

In Florida, lead arsenate sprays have been used to decrease the acidity of grapefruit, thereby advancing the harvest period (Harding and Fisher, 1945; Harding, 1946; Rygg and Getty, 1955; Deszyck and Ting, 1958). Tests have indicated that palatability of fruit is noticeably improved by lead arsenate treatment. Application of the sprays in the post-bloom period was found to reduce the acid content of fruit more in the Ruby Red than in the Marsh or Duncan grapefruit varieties. However, Rygg and Getty (1955) reported only a small, inconsistent reduction of acid content after use of arsenic sprays on grapefruit in Arizona and California.

In Florida, nonsprayed red grapefruit usually attain legal maturity during the last week of October. Deszyck and Ting (1958) found that a spray containing 0.4 pound of lead arsenate per 100 gallons resulted in legal maturity about one month earlier, or in the latter part of September. Higher concentrations resulted in slightly earlier legal maturity, but reduced acidity excessively for fruit picked late in the season. A concentration of 1.2 pounds of lead arsenate produced early maturity for the white varieties. Arsenic sprays were found to be more effective in wet seasons. In addition to reducing the acid content of grapefruit, arsenic has been found to raise the total sugar and bioflavonoid contents (Deszyck and Ting, 1960).

The second most abundant organic acid in citrus juice is malic acid (Sinclair, Bartholomew,

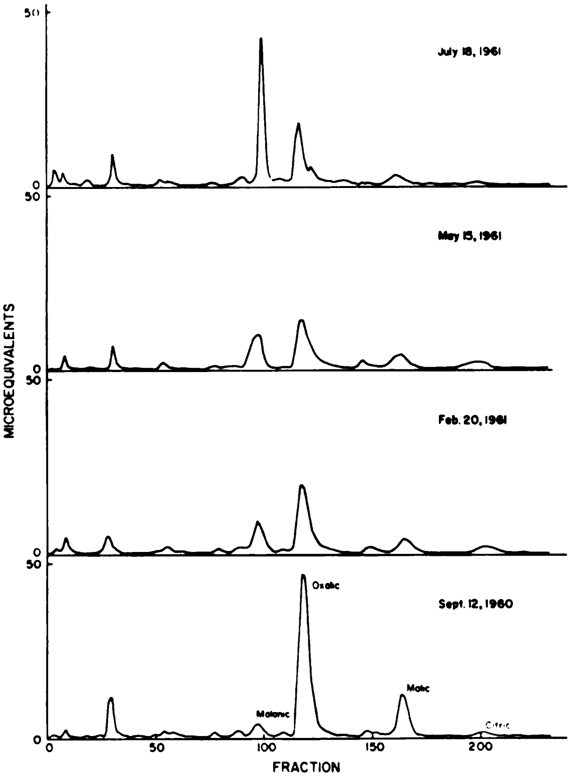


Fig. 2–13. Seasonal changes in flavedo acids of the Washington navel orange as reflected in silicic-acid column elution patterns (1 gm dry flavedo). (Clements, 1964*b*.)

and Ramsey, 1945). In oranges, it occurs in the range of about 1.4 to 1.8 mgm/ml. It is present in somewhat higher concentrations in lemons: 1.5 to 4.3 mgm/ml. Other acids of the citric acid cycle are present in lower concentrations than malic acid.

Sinclair and Eny (1947) reported that malic acid was the most abundant organic acid in citrus peel, followed by oxalic and citric as the second and third most abundant acids. Clements (1964*b*), however, employed chromatographic techniques and found that young Washington navel fruit had a very high oxalic acid content that decreased as the fruit matured. Oxalic acid did not fall below the malic acid content even after the normal picking season (fig. 2–13). Clements found malonic acid to be the second most abundant organic acid in the peel of mature Washington navel oranges. Malonic acid increased from a low level in the immature fruit to a level higher than malic acid late in the season after the usual harvest time. In view of the known specific inhibitory effect of malonate on succinic dehydrogenase, Clements (1964*b*) pointed out the possible importance of high malonate in the breakdown of citrus peel.

Vitamins and Other Growth Factors.—Numerous vitamins and other growth factors have been determined by Birdsall, Derse, and Tepley (1961) for lemons and Valencia and navel oranges (table 2–9).

Citrus fruits provide an important source of ascorbic acid for human nutrition. The juice of citrus contains about 40 to 70 mgm of ascorbic acid per 100 ml (Kefford, 1959). Like citric acid, ascorbic acid concentration has been found to usually decrease in the juice of oranges and grapefruit as they mature (Harding *et al.*, 1940; Harding and Fisher, 1945; Rygg and Getty, 1955), although the tendency is not universal (Harding *et al.*, 1940; Kefford, 1959).

Lemons, on the other hand, were found to have an increased ascorbic acid content during storage (Bartholomew and Sinclair, 1951), although the trend during maturation, as evidenced by analysis of fruit of three degrees of maturity at the beginning of storage, showed a downward tendency from dark green to light green to silver in rind color.

Only about one fourth of the ascorbic acid in citrus fruits is found in the juice. The remainder is in the peel, largely concentrated in the flavedo (Kefford, 1959; Birdsall *et al.*, 1961).

Inositol, a hexahydric cyclic alcohol, is abundant in both the peel and juice of oranges and lemons (Birdsall *et al.*, 1961) (table 2–9). Although this substance and choline are sometimes listed among the B vitamins, Fruton and Simmonds (1960) pointed out that neither has been found to be an essential cofactor in enzymatic reactions, and they should therefore not be included with the vitamins.

The carotenoids, on the other hand, are closely related to vitamin A. Some of them, such as β-carotene, have vitamin A activity and may properly be called provitamin A. Although it was formerly thought that a molecule of β-carotene could be split into two molecules of vitamin A, the current interpretation is that β-carotene is broken down from one end by β-oxidation to form a single molecule of vitamin A (MacKinney, 1961). Biotin, niacin, pantothenic acid, pyridoxine, riboflavin, and thiamine are present in the peel and juice of citrus in small amounts (table 2–9).

Carotenoids.—The carotenes and their oxygen-containing derivatives, the xanthophylls, constitute the bulk of the yellow, orange, and red

Table 2–9

COMPOSITION OF CALIFORNIA LEMONS, VALENCIA ORANGES, AND NAVEL ORANGES

Constituent	Method Reference*	Peel			Edible Portion			Juice		
		1957	1958	Average	1957	1958	Average	1957	1958	Average
				Lemons (Gm/100 Gm)						
Protein (Kjeldahl N × 6.25)	1	1.44	1.50	1.47	....	1.13	....	0.44	0.50	0.47
Amino nitrogen (Van Slyke)	....	0.18	0.22	0.20	....	0.05	....	0.03	0.04	0.04
Fat (ether extract)	1	0.25	0.34	0.30	....	0.32	....	0.16	0.22	0.19
Total soluble solids	....	8.90	10.47	9.69	....	8.82	....	7.97	8.63	8.30
Sugar:										
Total sugar	1	3.21	3.81	3.51	....	1.51	....	1.19	1.15	1.17
Sucrose		0.03	0.15	0.09	....	0.18	....	0.11	0.06	0.09
Reducing		3.18	3.66	3.42	....	1.33	....	1.08	1.09	1.09
Acid as anhydrous citric	....	0.41	0.38	0.40	....	4.93	....	5.91	6.05	5.98
Moisture	1	81.87	81.36	81.62	....	90.08	....	92.84	91.88	92.36
Ash	1	0.61	0.61	0.61	....	0.29	....	0.25	0.24	0.25
				Lemons (Mgm/100 Gm)						
Calcium	2	130.0	137.0	133.5	....	22.3	....	5.6	6.5	6.1
Magnesium	3	13.9	16.0	15.0	....	5.8	....	5.8	6.3	6.1
Iron	4	0.59	1.05	0.82	....	0.65	....	0.21	0.21	0.21
Phosphorus	5	12.3	12.7	12.5	....	15.8	....	9.6	10.9	10.3
Potassium	6	176	143	160	....	108	....	101	102	102
Sodium	6	6.6	6.4	6.5	....	2.5	....	1.0	1.0	1.0
Sulphur	1	11.0	12.7	11.9	....	5.3	....	2.0	3.3	2.7
Ascorbic acid:										
Reduced	7	138.0	120.0	129.0	....	52.7	....	44.8	42.7	43.8
Total ascorbic acid		....	....	....	....	54.7	....	....	....	....
Biotin	8	0.00262	0.00234	0.00248	....	0.00058	....	0.00028	0.00022	0.00025
Carotenoids:										
β-carotene	9	0.03	0.03	0.03	....	<0.01	....	<0.01	<0.01	<0.01
Total carotenoids		0.21	0.39	0.30	....	0.06	....	0.05	0.04	0.04
Choline	10	10.0	12.0	11.0	....	10.0	....	5.9	5.0	5.5
Folic acid	1	0.00548	0.00440	0.00494	....	0.00182	....	0.00094	0.00082	0.00089
Inositol	11	201	231	216	....	109	....	76	56	66
Niacin	1	0.358	0.354	0.356	....	0.129	....	0.069	0.072	0.071
Pantothenic acid	12	0.390	0.247	0.319	....	0.194	....	0.106	0.101	0.104
Pyridoxine	11	0.081	0.263	0.172	....	0.080	....	0.030	0.072	0.051
Riboflavin	1	0.057	0.100	0.079	....	0.021	....	0.013	0.011	0.012
Thiamine	13	0.067	0.048	0.058	....	0.042	....	0.029	0.032	0.031

Table 2–9 (Continued)

COMPOSITION OF CALIFORNIA LEMONS, VALENCIA ORANGES, AND NAVEL ORANGES

Constituent	Method Reference*	Peel			Edible Portion			Juice		
		1957	1958	Average	1957	1958	Average	1957	1958	Average
				Valencia Oranges (Gm/100 Gm)						
Protein (Kjeldahl N × 6.25)	1	1.75	1.31	1.53	1.31	0.95	1.13	1.13	0.88	1.00
Amino nitrogen (Van Slyke)	....	0.25	0.30	0.28	0.06	0.06	0.06	0.06	0.06	0.06
Fat (ether extract)	1	0.16	0.30	0.23	0.43	0.16	0.30	0.40	0.18	0.29
Total soluble solids	....	14.40	16.98	15.69	13.33	12.78	13.06	12.92	12.59	12.59
Sugar:										
Total sugar	1	7.22	7.87	7.55	8.70	9.49	9.10	9.90	9.54	9.72
Sucrose		2.04	1.93	1.99	4.14	4.68	4.41	4.91	4.56	4.73
Reducing		5.18	5.94	5.56	4.56	4.81	4.69	4.99	4.98	4.99
Acid as anhydrous citric	....	0.34	0.24	0.29	0.68	0.82	0.75	1.02	1.02	1.02
Moisture	1	71.77	73.26	72.52	85.05	85.41	85.23	86.99	87.27	87.11
Ash	1	0.82	0.74	0.78	0.51	0.45	0.48	0.31	0.37	0.34
				Valencia Oranges (Mgm/100 Gm)						
Calcium	2	157.0	164.0	161.0	36.1	37.3	36.7	8.0	11.0	9.5
Magnesium	3	20.3	24.0	22.2	11.3	11.6	11.5	10.1	11.4	11.3
Iron	4	0.79	1.00	0.80	0.68	0.86	0.77	0.35	0.31	0.33
Phosphorus	5	21.8	19.8	20.8	22.8	21.3	21.8	20.0	18.9	19.5
Potassium	6	240	183	212	182	163	173	164	162	163
Sodium	6	3.6	2.4	3.0	2.0	0.6	1.3	0.8	0.6	0.7
Sulphur	1	21.0	21.0	21.0	11.0	12.0	11.5	8.0	9.0	8.5
Ascorbic acid:										
Reduced	7	133.0	140.0	136.5	38.2	40.8	39.5	42.8	44.1	43.5
Total ascorbic acid		....	....	....	41.6	43.0	42.3	....	....	....
Biotin	8	0.00472	0.00548	0.00510	0.00122	0.00113	0.00118	0.00077	0.00081	0.00079
Carotenoids:										
β-carotene	9	....	0.25	0.25	....	0.15	0.15	....	0.17	0.17
Total carotenoids		6.6	13.2	9.9	3.2	3.6	3.4	2.5	3.2	2.8
Choline	10	25.0	21.0	23.0	8.7	14.5	11.6	7.2	8.8	8.0
Folic acid	1	0.01410	0.00950	0.01180	0.00495	0.00336	0.00416	0.00260	0.00320	0.00290
Inositol	11	267	246	257	205	203	204	182	137	159
Niacin	1	0.927	0.849	0.888	0.467	0.515	0.491	0.355	0.396	0.376
Pantothenic acid	12	0.593	0.386	0.490	0.311	0.240	0.276	0.245	0.168	0.207
Pyridoxine	11	0.221	0.130	0.176	0.066	0.063	0.065	0.066	0.047	0.057
Riboflavin	1	0.104	0.078	0.091	0.033	0.033	0.033	0.028	0.025	0.027
Thiamine	13	0.12	0.12	0.12	0.13	0.12	0.13	0.10	0.09	0.10

Table 2–9 (Continued)

COMPOSITION OF CALIFORNIA LEMONS, VALENCIA ORANGES, AND NAVEL ORANGES

Constituent	Method Reference*	Peel			Edible Portion			Juice		
		1957	1958	Average	1957	1958	Average	1957	1958	Average
					Navel Oranges (Gm/100 Gm)					
Protein (Kjeldahl N × 6.25)	1	1.56	1.56	1.56	1.19	1.25	1.22	0.94	1.06	1.00
Amino nitrogen (Van Slyke)	....	0.19	0.14	0.17	0.06	0.06	0.06	0.05	0.05	0.05
Fat (ether extract)	1	0.14	0.26	0.20	0.08	0.11	0.10	0.12	0.10	0.11
Total soluble solids	....	21.23	18.63	19.93	13.86	12.72	13.29	13.57	12.34	12.96
Sugar:										
Total sugar	1	10.30	10.05	10.18	9.74	8.99	9.37	11.30	9.45	10.38
Sucrose		0.81	0.90	0.86	4.48	4.17	4.33	5.72	4.70	5.21
Reducing		9.49	9.15	9.32	5.26	4.82	5.04	5.58	4.75	5.17
Acid as anhydrous citric	....	0.25	0.25	0.25	0.77	0.75	0.76	0.99	0.93	0.96
Moisture	1	75.68	74.50	75.09	84.65	85.56	85.10	86.45	87.32	86.89
Ash	1	0.66	0.63	0.65	0.37	0.48	0.43	0.36	0.45	0.40
					Navel Oranges (Mgm/100 Gm)					
Calcium	2	181.0	145.0	163.0	44.7	40.3	42.5	10.1	10.8	10.5
Magnesium	3	20.9	18.0	19.5	10.1	10.0	10.1	10.0	9.5	9.3
Iron	4	0.58	0.60	0.59	0.47	0.32	0.40	0.17	0.21	0.19
Phosphorous	5	16.4	15.4	15.9	21.5	20.8	21.2	17.8	17.8	17.8
Potassium	6	162	163	163	180	170	175	178	173	176
Sodium	6	2.0	1.8	1.9	0.7	0.6	0.7	0.6	0.5	0.6
Sulphur	1	15.0	16.1	15.6	9.1	9.2	9.2	6.1	7.0	6.6
Ascorbic acid:										
Reduced	7	242.0	201.0	222.0	60.1	54.0	57.1	58.7	59.9	59.3
Total ascorbic acid		....	....	....	61.7	56.2	59.0	....	....	....
Biotin	8	0.00294	0.00260	0.00277	0.00081	0.00062	0.00071	0.00061	0.00049	0.00055
Carotenoids:										
β-carotene	9	0.14	0.20	0.17	0.07	0.03	0.05	0.04	0.02	0.03
Total carotenoids		12.0	12.3	12.2	2.34	1.93	2.14	1.47	1.35	1.41
Choline	10	22.7	23.0	22.9	12.3	14.0	13.2	4.5	6.7	5.6
Folic acid	1	0.00890	0.00850	0.00870	0.00318	0.00360	0.00339	0.00159	0.00214	0.00187
Inositol	11	189	180	185	227	146	187	182	129	156
Niacin	1	0.624	0.704	0.665	0.453	0.501	0.477	0.430	0.428	0.429
Pantothenic acid	12	0.273	0.333	0.303	0.209	0.233	0.221	0.183	0.190	0.187
Pyridoxine	11	0.117	0.087	0.102	0.056	0.040	0.049	0.052	0.033	0.048
Riboflavin	1	0.101	0.089	0.095	0.047	0.043	0.045	0.037	0.031	0.034
Thiamine	13	0.09	0.09	0.09	0.10	0.09	0.10	0.10	0.10	0.10

Source: After Birdsall, Derse, and Teply (1961).

* Method references: (1) Association of Official Agricultural Chemists, 1955; (2) Ingols and Murray, 1949; (3) Harvey, Komarmy, and Wyatt, 1953; (4) Sandell, 1950; (5) Fiske and Subbarow, 1925; (6) Bills *et al.*, 1949; (7) Robinson and Stotz, 1945; (8) Wright and Skeggs, 1944; (9) Moore and Ely, 1941; (10) Horowitz and Beadle, 1943; (11) Atkin *et al.*, 1943; (12) Neilands and Strong, 1948; (13) Association of Vitamin Chemists, Inc., 1951.

Table 2–10

CAROTENOID COMPOSITION OF CALIFORNIA NAVEL ORANGE PULP AND PEEL

		Approximate Percentage of Carotenoid Mixture	
Fraction	Constituent	Pulp	Peel
I.	Hydrocarbons:		
	Phytoene	4.3	12.1
	Phytofluene	4.4	7.3
	α-Carotene	0.12	0.03
	P418*	0.11	....
	β-Carotene	0.5	0.15
	ζ-Carotene	8.5	8.4
II, A.	Monols:		
	Hydroxy-α-carotene	0.5	0.15
	Cryptoxanthin	10.0	3.1
II, B.	Monol Epoxides(?):		
	P352	....	0.15
	Cryptoxanthin 5,6,5′,6′-diepoxide	0.2	0.5
	Hydroxy-α-carotene 5,6-epoxide	....	0.10
	Cryptoxanthin 5,6-epoxide	0.4	0.7
	Hydroxy-α-carotene 5,8-epoxide	....	0.09
	Cryptoxanthin 5,6,5′,8′-diepoxide	....	0.04
	ψ-Cryptoflavin	0.4	4.7
	P399	....	0.26
III, A.	Diols:		
	Lutein	1.3	0.6
	Zeaxanthin	1.5	0.8
	Reticulataxanthin	....	0.26
III, B.	Monoepoxide Diols:		
	Antheraxanthin	11.6	3.1
	Flavoxanthin-like	....	0.16
	Mutatoxanthins	0.5	0.6
III, C.	Diepoxide Diols:		
	Violaxanthin	45.6	26.4
	Luteoxanthin	2.5	21.5
	Auroxanthin	....	0.3
IV.	Polyols:		
	Valenciaxanthin	2.2	1.3
	Sinensiaxanthin	2.2	2.7
	Trans-neoxanthin	0.5	....
	Valenciachrome	0.3	0.9
	Trolliflor-like	0.4	....
	Trollixanthin-like	1.2	0.8
	Sinensiaxanthin-like	0.3	1.8
	Trollichrome-like	0.17	....

Source: After Curl and Bailey (1961).

* P418 refers to substance with highest wavelength spectral absorption maximum at 418 mμ.

pigments found in citrus fruit peel and pulp. In detailed investigations of citrus fruit carotenoids, Curl (1953) and Curl and Bailey (1954, 1955, 1956, 1957*a*, 1957*b*, 1961) saponified the extracted pigments, separated them into several fractions by counter-current distribution, and then isolated the individual pigments by chromatography. By this procedure, more than thirty carotenoids have been identified in navel oranges (table 2–10).

The most generally abundant carotenoid in oranges and tangerines is violaxanthin. Other constituents accounting for 10 per cent or more of the carotenoid content in the peel or pulp of oranges, tangerines, and Ruby Red grapefruit are summarized in table 2–11. The Meyer lemon has been found to be a source of a number of unusual carotenoids (Curl, 1962).

The question of whether lycopene is synthesized in the fruit of Ruby Red grapefruit or translocated to it from the foliage was investigated by Purcell and Stephens (1959). They grafted immature Ruby Red grapefruit on the white Marsh variety, and, reciprocally, Marsh fruit on another red variety, Stanfield. The lack of accumulation of lycopene in the Marsh fruit nourished by leaves of a red variety, and a characteristic accumulation of lycopene by the Ruby Red fruit nourished by leaves of the white variety, indicated that the site of lycopene synthesis was in the fruit.

During maturation, both the pink (Thompson pink) and red (Ruby Red) grapefruit varieties were found to have a decreasing amount of lycopene and an increasing amount of β-carotene (Ting and Deszyck, 1958). Both pigments were more abundant in the red than in the pink variety.

Monselise and Halevy (1961) reported that a pink sport of the Shamouti orange (Sarah) contains lycopene and other unidentified carotenoids rather than anthocyanins, as found in the blood oranges.

During maturation of oranges, there is a loss of chlorophyll and an increase in carotenoids (Miller, Winston, and Schomer, 1940). In most instances, loss of chlorophyll is incomplete so that extracts of peel reveal at least trace amounts of the green pigments. Stearns and Young (1942) studied the coloring of several varieties of oranges and concluded that there was no definite color break until after exposure to minimum temperatures below 55°C. Under certain environmental conditions, there is a tendency for fruit to regreen in the spring and summer of the second season

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Table 2–11

VARIATIONS IN ABUNDANCE OF THE CONSTITUENTS WHICH ACCOUNT FOR AT LEAST TEN PERCENT OF THE CAROTENOIDS IN THE PEEL OR IN THE PULP OF ONE OR MORE OF THE CITRUS VARIETIES COMPARED

	Approximate Percentage of Carotenoids							
	Navel Orange*		Valencia Orange†		Blanco Tangerine‡		Ruby Red Grapefruit§	
Constituent	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Phytoene	4.3	12.1	4.0	3.1	5.8	4.2	16	47
Phytofluene	4.4	7.3	13	6.1	7.2	3.5	4.4	14
β-carotene	0.5	0.15	1.1	0.3	4.1	0.4	27	7.2
Lycopene	......	....	......	......	0.1	0.02	40	11
Cryptoxanthin	10.0	3.1	5.3	1.2	33	24	0.7	1.4
Antheraxanthin	11.6	3.1	5.8	6.3	9.7	6.2	0.7	......
Violaxanthin	45.6	26.4	7.4	44	14	24	0.9¶	1.0¶
Luteoxanthin	45.6	21.5	17	16	3.5	9.1	0.4	1.8
Auroxanthin	......	0.3	12	2.3	0.4	1.9	0.3	1.6

* Curl and Bailey (1961).
† Curl and Bailey (1956).
‡ Curl and Bailey (1957*b*).
§ Curl and Bailey (1957*a*).
¶ May include zeaxanthin.

(Caprio, 1956). Nitrogen fertilizer applied all or partly in the summer aggravates the tendency to regreen (Jones and Embleton, 1959).

Young and Erickson (1961) found that the greatest reduction in chlorophyll and greatest increase in carotenoids occurred in Valencia oranges exposed to a combination of cool day-air temperatures, cold night-air temperatures, and cold soil temperatures. When any of these temperatures was higher, the fruit tended to remain green in color (fig. 2–14 and table 2–12).

Flavonoids.—Most citrus flavonoids are glycosides in which the sugars, usually rhamnose and glucose, are attached to a flavonone. For detailed accounts of this group of compounds, the reader is referred to Horowitz (1961) and Kefford (1959).

Hesperidin is a common, tasteless flavonoid found in oranges, lemons, and some other species. Naringin is a bitter flavonoid found in grapefruit. Another bitter principle in citrus is limonin, which may cause bitterness in extracted juice, as in the navel orange (Kefford, 1959).

Interest in the flavonoids stems largely from their biological activity in the area of capillary permeability.

Water Content.—The water content of citrus fruits varies from about 70 to 92 per cent depending upon growth conditions and moisture availability. In California, citrus is grown near the coast, in intermediate valleys, in interior valleys, and in desert regions. These districts represent a wide range in humidity, temperature, and sunshine, which, in turn, influences fruit composition and physical characteristics. Fruit grown near the coast tends to have a more tender peel, a higher percentage of water in pulp and peel, and a higher percentage of minerals in the dry matter than fruit grown in hotter, drier, interior regions (Sinclair and Bartholomew, 1944; Monselise and Turrell, 1959; Turrell, Monselise, and Austin, 1964).

Table 2–13 lists moisture contents of peel and pulp of the four principal citrus varieties grown in California. Water in the peel falls in the range of 70 to 83 per cent. Water in the pulp is mostly in the range of 85 to 90 per cent. Although moisture in the peel of Valencia oranges varied greatly between coastal and desert areas, peel thickness varied little compared with differences in peel thickness found in Eureka lemons.

Bartholomew (1923), in determining the water content of lemon fruits at progressive stages of development, found that, although the total amount of water was much greater in mature than in young fruit, the percentage of water, based on green weight of fruit, increased comparatively little after fruit had attained a diameter of approximately 4 cm. Hodgson (1917), working with the orange, and Bartholomew (1926), working with the lemon, found that these fruits are good illustrative examples of how the leaves of a plant may withdraw water from the fruits when

Table 2–12

MATURITY OF FRUIT AND ABSORBANCE OF PEEL EXTRACT OF CALIFORNIA VALENCIA ORANGES MADE FROM FRUIT EXPOSED TO VARIOUS TEMPERATURE CONDITIONS. MEANS BASED ON FIVE FRUIT EACH*

				Absorbance of Pigments				
				Unseparated		Separated by Chromatography		
Temperature (°C)							Xanthophyll Layers 435 Mμ	
Day-time Air	Night-time Air	Soil	Maturity (Ss/Acid)	Total 445 Mμ	Chloro-phyll 665 Mμ	Carotene 445 Mμ	Less Adsorbed R_f—.18	More Adsorbed R_f—.08
Experiment I. (started January 4, 1960—harvested February 19, 1960)								
20	12	20	5.25 a	0.456 b	........†	0.126 a	0.250 c	0.375 b
20	7	20	4.64 b	0.573 b	0.092 a	0.098 b	0.334 bc	0.460‡
20	12	12	5.42 a	0.061 b	0.029 b	0.085 a	0.475 b	0.275 b
20	7	12	5.19 a	0.843 a	0.005 c	0.083 a	0.876 a	0.836 a
Experiment II. (started February 26, 1960—harvested April 12, 1960)								
20	20	20	6.83 a	0.196 b	0.041 a	0.052 a	0.070 c	0.164 c
20	15	20	7.42 a	0.330 b	0.020 b	0.049 a	0.242 b	0.316 b
20	20	12	7.59 a	0.273 b	0.020 b	0.034 a	0.166 bc	0.249 bc
20	15	12	7.23 a	0.543 a	0.010 b	0.066 a	0.391 a	0.583 a
Experiment III. (started April 18, 1960—harvested May 25, 1960)								
30	12	20	8.93 a	0.165 a	0.013 a	0.040 a	0.107 a	0.177 a
30	7	20	9.34 a	0.213 a	0.013 a	0.049 a	0.130 a	0.232 a
30	12	12	9.07 a	0.189 a	0.016 a	0.051 a	0.114 a	0.256 a
30	7	12	9.62 a	0.234 a	0.021 a	0.045 a	0.154 a	0.215 a
Greenhouse fruit harvested								
February 28, 1960			5.02 a	0.370 a	0.170 a	0.110 a	0.045 a	0.161 a
April 21, 1960			7.91 b	0.253 b	0.028 b	0.068 b	0.105 b	0.183 ab
May 31, 1960			9.61 c	0.214 b	0.004 c	0.041 c	0.135 c	0.253 a

Source: After Young and Erickson (1961).

* Means followed by the same letter are *not* significantly different. Means not followed by the same letters are significantly different at the 5% level, as determined by multiple range testing.

† From observation, these fruit were as green or greener than any others in the experiment.

‡ Mean of three fruits.

needs of foliage for water cannot be supplied through the roots.

Bartholomew found that during summer months, under average southern California conditions, lemon leaves begin to withdraw water from the fruits at about 6 to 7 A.M. and continue to do so until about 5 to 6 P.M. Figure 2–15 illustrates to what extent the fruits may be drawn upon to relieve a water deficit in leaves when water is not readily available from another source. Rokach (1953) demonstrated that Shamouti oranges contribute water to leaves during periods of water deficit and that water was supplied to the leaves mainly by the peel. Although young Shamouti oranges have a much lower density of stomata than the leaves, it was found that the transpiration rate was about the same as for leaves per unit of surface area.

Photosynthesis

The flavedo of young citrus fruit contains chlorophyll, which functions photosynthetically and contributes to the light fixation of carbon dioxide. Todd, Bean, and Propst (1961) measured the photosynthetic activity of developing fruits, including oranges and lemons, and found that in sunlight the fixation of carbon dioxide was about equal to its release from respiration. In other words, photosynthesis approached the compensation point in high light intensity. Under fluorescent lamps, the photosynthetic rate was much below the rate of respiration. They concluded that

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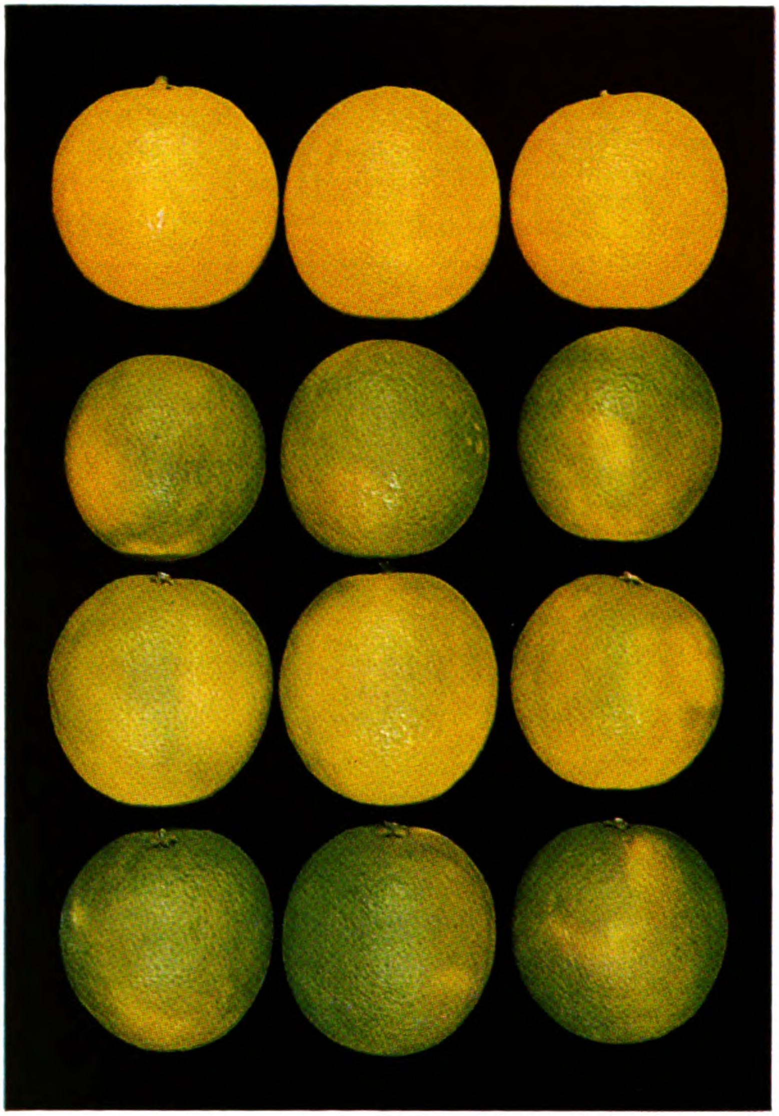

Fig. 2–14. Effects of air and soil temperatures on the coloring of Valencia oranges. Illustration of fruit in Experiment I, table 2–12. All fruits were exposed to a daytime air temperature of 20°C. The two upper rows were exposed to a nighttime air temperature of 7°C, and the two lower rows were exposed to a nighttime temperature of 12°C. The top and third rows had a soil temperature of 12°C, and the second and fourth rows had a soil temperature of 20°C. Note that the most highly colored fruit occurred where both the nighttime air and soil temperature were low (top row), and that a higher soil temperature (second row from top) or a higher nighttime temperature (third row from top) resulted in markedly less fruit coloring.

Table 2–13

MEAN PEEL THICKNESS AND MOISTURE CONTENTS OF PEEL, PULP, AND WHOLE FRUIT OF FOUR VARIETIES OF CITRUS GROWING IN VARIOUS CLIMATIC DISTRICTS IN CALIFORNIA

Fruit-Growing District	Peel Thickness (Mm)	Moisture (Per Cent) Peel	Pulp	Whole Fruit
Eureka Lemon				
Coastal	7.3	81.7	88.6	84.7
Interior	4.7	79.5	89.0	85.4
Desert	3.9	79.1	88.5	85.5
Marsh Grapefruit				
Intermediate	5.8	83.4	90.7	88.2
Interior	4.9	79.4	89.1	86.3
Desert	5.5	75.0	87.4	83.8
Valencia Orange				
Coastal	4.1	76.4	87.4	84.5
Interior	3.5	70.6	84.6	81.5
Desert	4.1	70.3	86.2	82.3
Washington Navel Orange				
Intermediate	5.7	80.1	88.4	85.4
Interior	5.7	75.6	87.4	83.7

Source: Adapted from Turrell, Monselise, and Austin (1964).

although photosynthesis occurs in green, developing fruits, it accounts for only a small part of the requirement for fruit development. The rate of photosynthesis per unit area of fruit surface decreased slowly as the fruit developed. On the other hand, respiration decreased rapidly per unit of fruit weight as fruit increased in size. The reason for placing the photosynthetic rate on a unit of surface area and the respiration on a unit of fruit weight was because of the surface distribution of the chlorophyll while respiration was occurring in all of the tissues.

The photosynthetic rates of oranges and lemons containing various amounts of chlorophyll was found to be roughly proportional to chlorophyll content for a given variety (Bean, Porter, and Barr, 1963). However, green lemons, which contain less chlorophyll than green oranges, showed a higher rate of photosynthesis than oranges. Fully orange-colored oranges and fully yellow-colored lemons were found to have small amounts of chlorophyll which showed a proportional photosynthetic activity as measured by fixation of $C^{14}O_2$.

Bean and Todd (1960) studied photosynthetic and respiratory activity of developing Valencia oranges in intact fruit and in tissue preparations by means of $C^{14}O_2$. In the light, carbon dioxide fixation by the flavedo was many times greater than in the dark, both in intact fruit and tissue preparations, indicating considerable photosynthetic activity (table 2–14).

By contrast, the albedo and vesicles fixed about the same amount of carbon dioxide in light as in dark, reflecting the near or complete absence of chlorophyll in these parts of the fruit. However, Bean and Todd found a small amount of labeled sucrose in albedo and flavedo tissue preparations exposed to light, but not in tissues kept in the dark. They interpreted this as evidence of slight photosynthetic activity by these tissues (table 2–15). Although all parts of the fruit showed dark fixation of carbon dioxide, the vesicle tissue preparations showed an extraordinary amount that represented from one fourth to one third of that fixed through photosynthetic activity of the flavedo in light. Carbon dioxide fixation in vesicles of intact fruit was much less than in vesicles of tissue preparations, suggesting that the rate was limited by an inadequate supply of la-

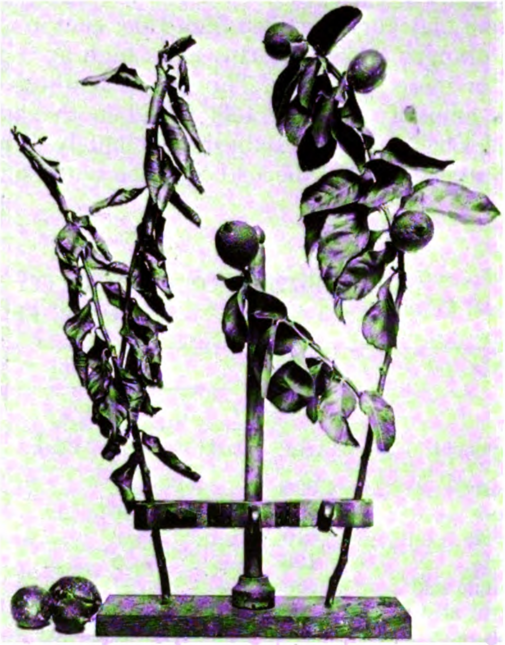

Fig. 2–15. Illustration of the degree to which the fruits may be called upon to relieve a water deficit in the leaves. Photograph was taken forty-eight hours after the branches were cut and arranged as shown. Detached fruits were comparatively firm, whereas the attached ones had become noticeably flaccid. (Bartholomew and Reed, 1943.)

Table 2–14

C^{14}-ACTIVITY[*] IN ALCOHOL-SOLUBLE AND ALCOHOL-INSOLUBLE FRACTIONS OF ORANGE TISSUES EXPOSED TO $C^{14}O_2$

	Alcohol-Soluble Extract†				Alcohol-Soluble Residue			
	Intact Fruit		Tissue Preparation		Intact Fruit		Tissue Preparation	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Flavedo	34,700	1,540	54,000	3,700	15,400	280	6,670	490
Albedo	3,400	1,150	2,500	1,950	235	160	280	280
Vesicle	2,580	2,500	13,600	16,500	284	360	2,580	1,680

Source: Bean and Todd (1960).

[*] Activity is given as counts per minute per milligram of the dried soluble solids or of the alcohol-insoluble residue. Counting was done to within 3% statistical error.

[†] Total activity in the various extracts from intact fruits: light (10^5 cpm): flavedo, 165; albedo, 18.0; vesicles, 11.0; dark (10^5 cpm): flavedo, 6.9; albedo, 5.7; vesicles, 7.4.

beled CO_2 diffusing into the fruit. In this regard, Eaks and Ludi (1960) found that the internal atmosphere of Valencia oranges was under positive pressure, indicating rapid exchange of gas did not occur.

With the exception of mutants like Faris sweet lemon, citrus fruits characteristically accumulate citric acid in the juice vesicles. The acid content may vary from less than 1 per cent in mature oranges and mandarins to as high as 6 per cent or more in lemons. The site of synthesis of large amounts of citric acid found in the juice vesicles has not been established definitely. It has been suggested that the acid could be synthesized in the vesicles where it is found (Huffaker and Wallace, 1959; Bean and Todd, 1960) or it could be translocated from other parts of the plant, such as roots, leaves, and fruit peel, where organic acid synthesis has been demonstrated (Huffaker and Wallace, 1959).

The latter authors proposed that the dark fixation of CO_2 is part of a major pathway of citric acid synthesis in citrus fruit vesicles (fig. 2–16). The steps leading to citrate formation may be traced from the oxidation of sugars to pyruvate, thence to formation of phosphoenolypyruvate (PEP) or to a decarboxylation forming acetyl-CoA. Carbon dioxide is incorporated in the pres-

Table 2–15

DISTRIBUTION OF C^{14} ACTIVITY IN COMPONENTS OF ALCOHOL-SOLUBLE EXTRACTS FROM ORANGE TISSUES[*]

	Intact Fruit						Tissue Preparations					
	Flavedo		Albedo		Vesicles		Flavedo		Albedo		Vesicles	
Component	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Glucose	1,020	......	....	......	......	......	1,290	......	......	......	....	....
Sucrose	10,400	......	1,140	......	......	......	9,870	......	159	......	146	....
Fructose	810	......	....	......	......	......	860	......	......	......	....	....
Alanine	100	......	5	......	+	+	330	......	+	......	....	....
Glutamic acid	1,200	+	37	+	+	+	610	13	+	......	37	+
Aspartic acid	+	90	10	55	275	190	160	40	35	23	182	209
Serine	180	65	45	100	200	185	120	70	135	110	146	101
Malic acid	860	435	45	57	325	355	950	420	141	120	1,210	1,340
Citric acid	30	......	30	52	460	630	33	20	......	......	475	445
Other acids†	+	+	+	+	160	160	67	67	88	+	273	283
Phosphate esters	+	......	....	......	......	......	87	67	......	......	30	....

Source: Bean and Todd (1960).

[*] Activity given throughout as counts per minute per milligram of dried soluble extract. Activity was neglible or too low for measurement where no figure is given.

[†] Succinate and fumarate were lost by running off the edge of the paper. Generally they were of low activity.

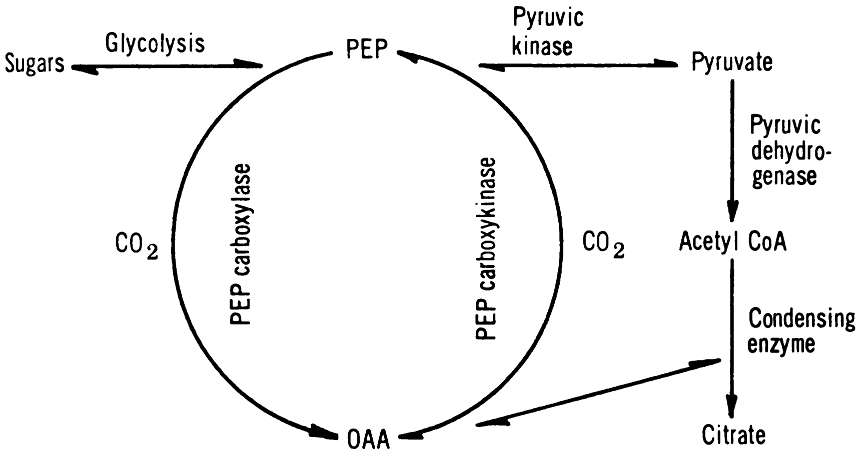

Fig. 2–16. A proposed major pathway of citric acid synthesis in citrus fruit vesicles. (Huffaker and Wallace, 1959.)

ence of PEP carboxylase or PEP carboxykinase to form oxaloacetic acid (OAA), which then condenses with acetyl-CoA to form citrate.

Sekhara Varma, and Ramakrishnan (1956) attributed the accumulation of citric acid in citrus fruit to a block in the aconitase activity which interferes with conversion of citrate to aconitic acid.

Clark and Wallace (1963) showed that the CO_2-fixing ability of the fruit did not determine the extent to which organic acids accumulated in those fruits. For example, sweet lime exhibited higher dark fixation of CO_2 than lemon or orange fruits but had the lowest acidity. Lemon, on the other hand, had the highest acidity but the lowest ability to fix CO_2.

Maturation

Sweet varieties of citrus are edible several weeks or even months before they reach full maturity. This slow maturation in the final stages of development is in marked contrast to many other fruits where final changes occur in a few days.

The slow accumulation of sugars and decrease in acid content imperceptibly alter maturity of oranges from day to day. Furthermore, all fruits on a given tree are not at the same stage of maturity at one time. For many fruits other than citrus, color differences are useful for selective or "spot" picking, particularly where earliness is an important economic factor or uneven ripening necessitates this practice. In California, grapefruit, mandarins, and oranges cannot be spot-picked by any known criterion of color, although some spot-picking of Washington navel oranges is done for the sake of the peel color itself. However, in Florida, a correlation between color and the solids-acid ratio has been shown for oranges (Sites and Reitz, 1950*a*).

Lemons and limes—the highly acid forms of citrus—are desired for their acidity, and therefore ripeness is not a factor in time of picking. Color is useful for spot-picking lemons and limes because it reveals important information about fruit maturity. Eureka lemon trees set fruit several times during the year in mild climatic coastal areas of California. This habit necessitates harvesting lemons several times each year. As a result, color becomes an important criterion for spot-picking. A lemon which is dark green in color with a rough peel has not attained full size. If such a dark-green lemon is of desirable marketing size, it may be picked to prevent it from becoming too large for acceptability and also because it is known that fruit that is less ripe at the time of picking has a longer storage life. Although dark-green lemons are not fully developed, they may be mature enough to complete the necessary changes in storage and cure into high quality fruit.

The lemon attains nearly maximum maturity and size about the time color begins to change from green to yellow. Prior to this change, the dark-green color changes to a lighter green and the peel usually assumes a smoother texture. Lemons in this stage of development are considered in their prime for picking. They can be degreened quickly in storage and marketed soon afterwards, or they can be stored for several months awaiting increased demands for fruit. Degreening lemons in storage is preferable to degreening on the tree, since results in the packinghouse are more complete and uniform. Probably this is because of the constant low ethylene content in the atmosphere of storage rooms, emitted mostly by decaying fruit. If rapid degreening is desired, additional ethylene may be liberated in special degreening rooms.

Another reason for picking lemons before they are tree ripe (yellow) is that resistance to decay is greater when lemons are green, tending to increase their storage life. A discussion of the use of growth regulators for increasing the storage life of lemons may be found in Chapter 6.

In California, the basis for legal maturity of oranges is a ratio of 8-to-1 for total soluble solids to titratable acidity. The balance between sugars, which accounts for about 75 per cent of the total soluble solids, and the sourness produced by acidity is the best criterion in correlating fruit quality with consumer acceptance. Although most people prefer a sweeter-tasting orange than the 8-to-1 ratio provides, enough consumer acceptance exists at this level to make the standard suitable for early harvesting. If all oranges were harvested at the minimum legal standard of maturity, consumption of fresh oranges would undoubtedly decline. Probably the existing standard can be retained only because harvesting of the crop is spread over an extended period, permitting additional ripening of the fruit.

Presently, the degree of maturity of oranges is based on the ratio of a composite sample. Thus, some oranges in a lot of fruit that is barely over the 8-to-1 ratio have a ratio of less than 8-to-1. When attempting to supply fruit for an early market, it would obviously be advantageous to avoid picking underripe fruit by some program of spot-picking.

With this in mind, Sites and Reitz (1949, 1950*a*, 1950*b*) made a study in Florida of the maturity of individual oranges in regard to tree position. They analyzed individually all oranges from a tree and correlated various measurements with position on the tree. The percentage of soluble solids was highest for fruit in exposed locations (i.e., outside fruit). Fruit in the tree canopy was intermediate, whereas inside fruit had the lowest percentage of soluble solids. In addition, there was an increase in solids with increased height of fruit on the tree. This characteristic of fruit composition was clearly correlated with exposure to light. The fruit was also graded by Sites and Reitz for color according to the same tree locations. Green fruit was associated with an inside location, low soluble solids, and low vitamin C; yellow fruit with partially shaded conditions, intermediate solids, and intermediate vitamin C; and orange fruit generally with exposed conditions, highest soluble solids, and highest vitamin C.

Although light may be an important factor in degreening fruit, other data indicate that color is also dependent on degree of maturity at the time of exposure to low temperatures. Very probably full exposure of leaves attached to stems bearing fruit is important for maximum production of soluble solids, which then results in greater translocation of solids to the fruit.

The average percentage of titratable acid in fruit from various locations on the tree did not differ much as compared with soluble solids (Sites and Reitz, 1949, 1950*a*). However, the northeast section of the tree seemed to have less acid fruit than other exposures. Individually, fruits had a wide range in acid concentration, the highest value being more than two and one half times the lowest. The soluble solids-to-acid ratio varied in the same manner as soluble solids in that the outside fruit had the highest average ratio, canopy fruit the next highest, and inside fruit the lowest. Also, the ratio increased with height on the tree.

The vitamin C content in the juice of Valencia oranges was found to vary with exposure of fruit in much the same manner as the soluble solids (Sites and Reitz, 1950*b*). Outside fruit had the highest vitamin C content, canopy fruit the next highest, and inside fruit the lowest. In all categories, vitamin C content increased with increased height of fruit in the tree. Juice content of fruit, however, tended to decrease with increased height and exposure.

The study indicated that under conditions such as those in Florida it might be possible to use the color of peel and location of fruit on the tree to spot-pick for earliness.

PHYSIOLOGICAL DISORDERS OF FRUIT

Splitting

The splitting of citrus fruits is probably much more frequent in Washington navel oranges than in other varieties. The disorder is physiological, but no single factor has been found to be responsible for splitting.

In Washington navel oranges, splitting usually begins in the peel at the stylar end of fruit,

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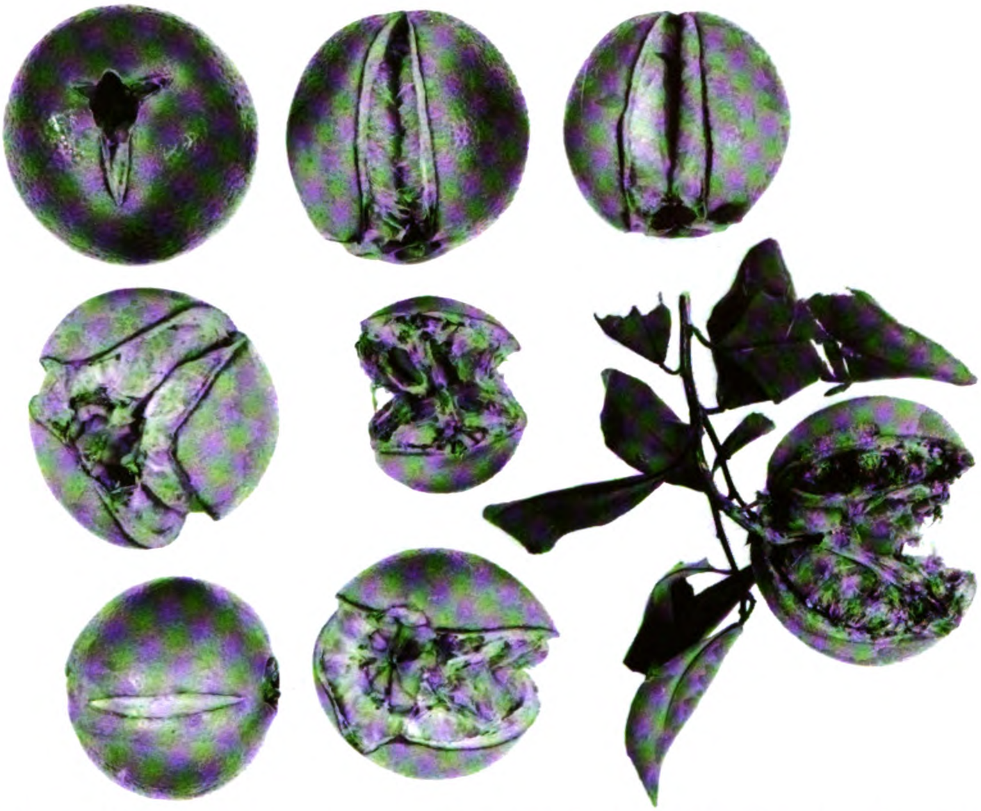

Fig. 2–17. Split Washington navel oranges. Most of the splitting originates at the navel or stylar end of the fruit as one or more fissures. In occasional fruits, the split originates between the ends. (Erickson, 1957.)

although it may occasionally originate midway between the ends (fig. 2–17). The peel seemingly fails to enlarge as fast as the fruit interior and therefore develops one or more fissures which may be short or extend the length of the fruit. Cook (1913) described splitting of navel oranges as "probably caused by spasms of growth caused by irregularities in culture, irrigations, or seasons." Coit (1915) developed a theory that splitting was caused by a combination of fluctuating environmental factors such as soil moisture, temperature, and relative humidity, and a thinness of peel at the stylar end of split fruit which produced differentiated growth in peel and pulp.

Erickson (1957) compared split and normal Washington navel oranges and found that water contents of peel and pulp were lower in split fruit. Phosphorus and nitrogen were significantly higher in the peel of split fruit, while calcium, potassium, magnesium, and sodium were no different than in normal fruit. Only calcium was higher in the pulp of split fruit.

Granulation

In California, granulation is generally considered to be a physiological disorder of citrus most commonly found in Valencia oranges. Grapefruit is less often subject to granulation. Granulation in grapefruit is frequently spoken of as "ricing." Some varieties of mandarins are also subject to granulation.

Granulation is characterized by enlarged, hardened, and nearly colorless juice vesicles which first appear at the stem end of the fruit (fig. 2–18). In badly granulated fruit, one third to one half of the juice vesicles may be affected (Bartholomew, Sinclair, and Turrell, 1941). Cell walls in granulated juice vesicles are thicker than normal, and there are differences in cell constituents. The pectic substances increase in amount and form gels. The alcohol-insoluble fraction of juice vesicles increases with the severity of granulation and shows a high correlation with the hardness of vesicles (fig. 2–19). Sugars, organic acids, and carotenoids decrease, whereas mineral

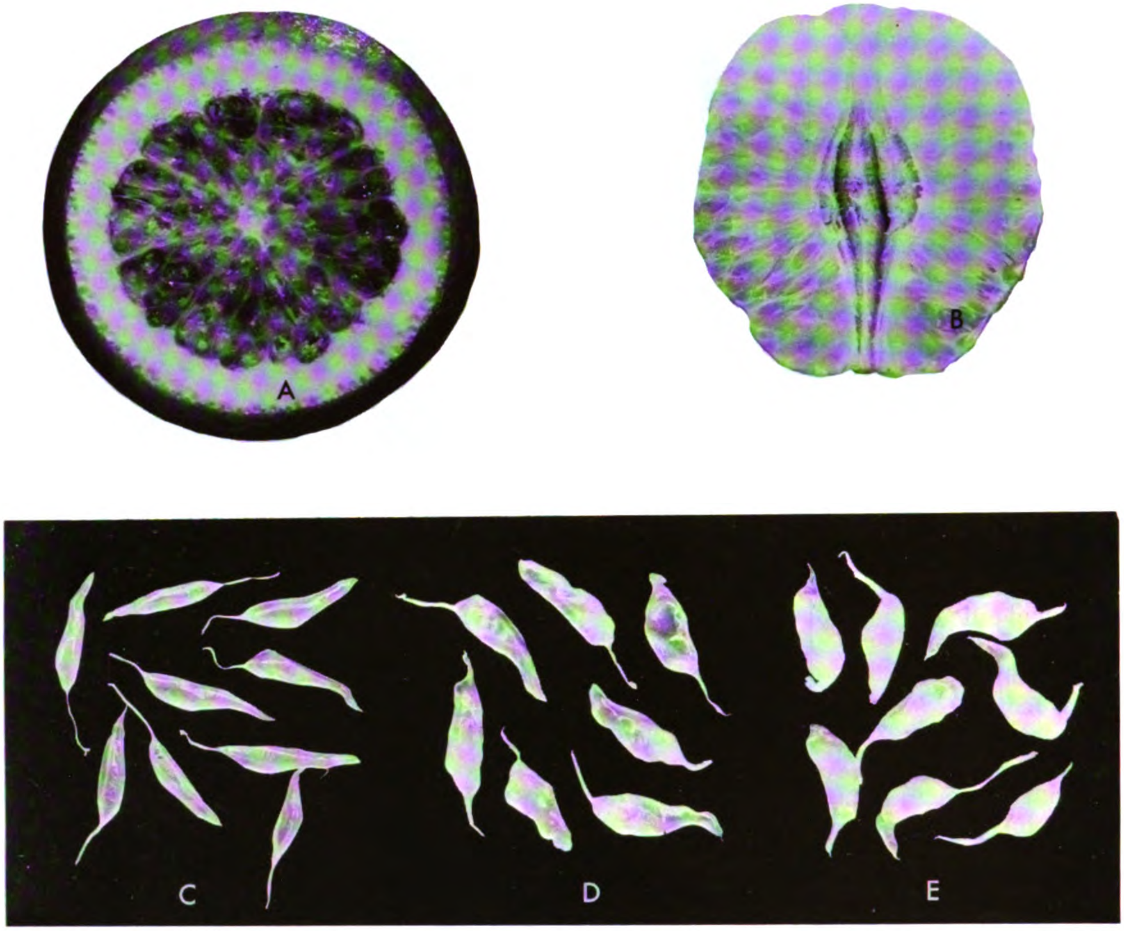

Fig. 2–18. Granulation in a Valencia orange. *A*, granulated vesicles as seen near the stem end of the fruit; *B*, pulp of a partially granulated fruit (note grayish appearance of upper or stem-end portion); *C*, healthy vesicles; *D*, and *E*, partially and wholly granulated vesicles. (Bartholomew and Reed, 1943.)

constituents increase as vesicles become granulated (Sinclair and Joliffe, 1961).

Although moisture content of granulated vesicles is higher than normal, the water is bound in gels and therefore not available as juice. Because of the low sugar and organic acid content, granulated vesicles are rather tasteless. In late stages of granulation, cells in the interior of juice vesicles may collapse, leaving gas-filled cavities.

The cause of granulation has not been determined. However, several factors have been studied to determine their influence on its occurrence. Young, vigorous trees are more likely to develop granulated fruit than older trees. Large fruits have more granulation than small ones (fig. 2–20). Granulation increases as the picking season advances. It is more prevalent near the coast than inland in California. High soil moisture is more conducive to granulation than low soil moisture.

Rootstocks vary in the amount of granulated fruit produced, but no rootstock avoids it. Bartholomew *et al.* (1941) noted that variation in granulation occurred from tree to tree. Some trees regularly tended to have more granulated fruit than others. This suggested that granulation was a condition inherent in the tree.

Picking large fruit early in the season before granulation becomes serious has been recommended as a measure to lessen granulation. Lime sprays seemed to reduce granulation, but the amount of lime required is both expensive and hazardous to the tree. A reduction in irrigation has also been suggested as a means of lessening granulation, but it lowers fruit yield.

A clear-cut reduction of granulation was found on the Limoneira Ranch in Santa Paula, California, after the use of 2,4-dichlorophenoxyacetic acid (2,4-D) for increasing fruit size. During packing operations early in September, 1951, it was noted that culling was extensive in nonsprayed Valencia oranges as compared with 2,4-D treated fruit. The author made further observations confirming the response to the spray (table 2–16). He concluded that the known effect of 2,4-D in prolonging the growing period of fruit was responsible for retarding granulation.

Dry juice sac originally was considered to be a phase of granulation. However, the condition of the juice sacs and the random occurrence of this phenomenon in fruit of all sizes led Bartholomew *et al.* (1941) to conclude that dry juice sac had a different origin than granulation. Only in the respect that dry juice sac occurs late in the season is it similar to granulation. Instead of swelling and hardening, as in granulation, vesicles of dry juice sac shrivel and dry out, particularly after removal of fruits from the tree. In contrast, granulation does not worsen after fruit is picked.

Endoxerosis

Endoxerosis is a physiological disorder of lemons in which there is deterioration of tissues beginning at the stylar end of the fruit. Internal decline, blossom-end decay, and tip deterioration

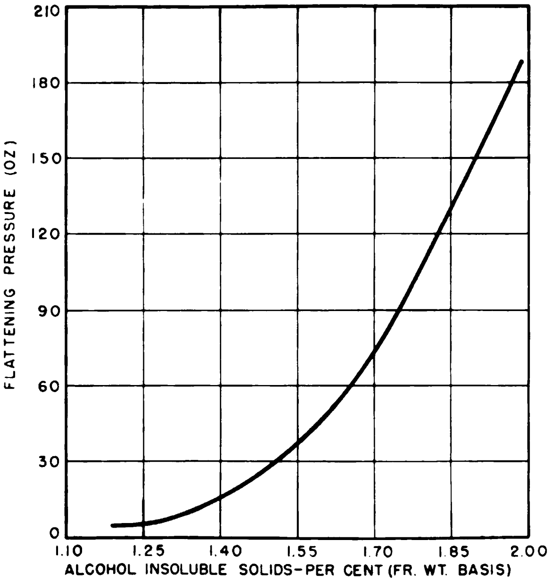

Fig. 2–19. Association between tenderometer readings (flattening pressure in oz.) and the alcohol-insoluble solids of granulated juice vesicles. (Sinclair and Jolliffe, 1961.)

Table 2–16

GRANULATION IN CALIFORNIA VALENCIA ORANGES FROM CONTROL TREES AND TREES SPRAYED ON JULY 1, 1950 WITH 16 PPM OF 2,4-D TO INCREASE FRUIT SIZE

Date of Sampling	Amount of Granulation*	Treatment Control (Per Cent)	 Sprayed (Per Cent)
Sept. 14, 1951†			
	None	18.0	37.0
	Slight	22.0	33.0
	Moderate	35.0	19.0
	Severe	25.0	11.0
		100.0	100.0
Sept. 26, 1951‡			
	None	29.0	30.2
	Slight	20.5	25.0
	Moderate	27.5	26.8
	Severe	23.0	18.0
		100.0	100.0
Average			
	None	23.5	33.6
	Slight	21.2	29.0
	Moderate	31.3	22.9
	Severe	24.0	14.5
		100.0	100.0

* None: no granulated vesicles.
Slight: 1 to 3 granulated vesicles at stem end of fruit (first cut of fruit).
Moderate: granulated vesicles showing in second cut (½ to ¾ inch below first cut).
Severe: granulated vesicles showing in third cut.

† Mean diameter of control fruit = 75.2 ± 3.3 mm (stand. dev.)
Mean diameter of 2,4-D-treated fruit = 75.4 ± 3.3 mm.

‡ Mean diameter of control fruit = 74.7 ± 4.6 mm.
Mean diameter of 2,4-D-treated fruit = 75.1 ± 4.2 mm.

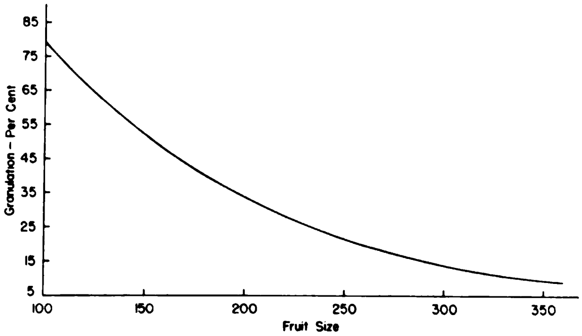

Fig. 2–20. Percentage of granulation, based on the number of fruits affected, in relation to fruit size. Sizes are shown as number of fruit per standard wooden box. (Sinclair and Jolliffe, 1961.)

are other names that have been applied to the phenomenon. Membranous stain and browning of the core, however, are considered to be distinct from endoxerosis (Bartholomew, 1937).

Endoxerosis is a common phenomenon in California lemon groves in summer, particularly in the hot inland valleys. Endoxerosis is least serious near the coast where it may affect as much as 60 per cent of the fruit of a given pick.

Trees of all ages have endoxerotic fruit, but young, thrifty trees with heavy foliage usually have a much higher percentage than trees with less dense foliage. The fruit is usually not affected until it is well advanced in development. Most endoxerotic fruit is yellow at the time of deterioration, although silver and green fruits are also affected.

The first sign of endoxerosis in the lemon is formation of cavities adjacent to the vascular bundles in the stylar region of the peel (Bartholomew, Barrett, and Fawcett, 1923). After formation of the cavities, a colorless, gummy substance is exuded which may clog some of the xylem vessels. Gum also forms on the twigs to which affected fruits are attached (Bartholomew, 1928*a*). Potometer tests of twigs bearing endoxerotic fruit show a reduced ability to translocate water (Bartholomew, 1928*b*).

As endoxerosis progresses, juice vesicles near the stylar end of the fruit become affected, lose water, and collapse. These changes are not readily evident from the fruit exterior. Frequently, a dull appearance develops, however, and the greenish fruits color prematurely at the stylar end. Also, peel may be sunken somewhat, thus indicating internal deterioration. External signs may permit from 50 to 90 per cent culling of the fruit (Bartholomew, 1937). Desiccation lowers specific gravity of the fruit and permits its separation in the washer on the basis of buoyancy. When as much as a third of the pulp tissues are affected, the fruit usually abscises from the tree.

Endoxerosis occurs in June or July and is evident until September or October, although it has been reported as early as March and as late as November (Bartholomew, 1937).

Lemon fruits are very sensitive to changes in water content of leaves, as affected by soil moisture and climatic conditions. The fruits show the effect of a water deficit long before leaves wilt. Under arid conditions, lemon trees may not be able to obtain adequate water to supply demands even when soil is moist. A diurnal moisture deficit was found to be reflected in diurnal fluctuations of fruit size (Bartholomew, 1926). In experiments where water relations of trees were altered, less endoxerosis developed (Bartholomew, 1937). For example, maintaining more moisture in the soil, covering trees with cheesecloth, and partially defoliating trees were various methods of reducing water deficit in the trees and fruits. Under these conditions, less endoxerosis developed, although satisfactory control was not attained.

Spoehr (1919) concluded that continued low water content of the tissues and high temperature are conducive to the transformation of monosaccharides into pentosans. Bartholomew and Robbins (1926) found such conditions also favor formation of endoxerosis in lemons. The ratio of hexosans to total carbohydrates was always found to be less in endoxerotic than in healthy lemons of the same lot, whereas the ratio of pentosans to total carbohydrates was found to be greater. The ratio of pentosans to hexosans was much greater in endoxerotic fruits. Endoxerotic tissues contained from 39 to 63 per cent more pentosans than healthy tissues. Results indicated that the pentosans, and hence gumming, were formed at the expense of the hexosans.

Creasing

Creasing in oranges is a defect of the peel which is manifested by the appearance of random grooves on the surface of the fruit (fig. 2–21). Badly creased fruit is unattractive and its fragility presents packing and shipping problems. The flavedo of creased fruit becomes depressed in areas where the albedo breaks and cracks. In early stages of creasing, there may be merely the development of lucunae in the albedo (fig. 2–21), whereas in advanced stages the fruit may be split (Miller and Turnbull, 1948).

In general, the amount of creasing is greater as the season advances. Analysis of creased and noncreased fruits from the same trees indicated that the former may be more mature, as revealed by the higher soluble solids-to-acid ratio in the creased fruit (Jones *et al.*, 1967). Creasing has been found to be associated with high crop loads. In addition, the higher the proportion of fruit on a tree affected with creasing, the greater is the severity of creasing on individual fruits.

In southern California, more creasing occurs on fruit on the south side of the tree, and creasing develops more on the side of the fruit

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facing the trunk. Because the incidence of creasing varies from year to year on the same trees, other climatic factors appear to be involved than those connected with the orientation and exposure of trees and fruit.

Small mature fruits are more subject to creasing than large ones (Miller, 1945), which may be related to the thinner peel usually associated with smaller fruit. Cultural practices which produce thick peel tend to reduce the incidence of creasing, whereas practices that produce thin peel have the opposite effect. Thus, the use of much fertilizer, particularly potassium and nitrogen, produces coarser, thicker peel, less subject to creasing. Use of phosphorus or no fertilizer tends to produce fruit with smooth, thin peels that are prone to creasing (LeRoux and Crous, 1938; Haas, 1950; Fourie, 1957; Jones *et al.*, 1967).

Although climatic factors and mineral nutrition both seem to influence creasing, the basic cause of the disorder has yet to be established.

AIRBORNE PHYTOTOXICANTS

Citrus accumulates fluorine poorly when an atmospheric source is absent. The amount of fluorine found in citrus leaves in areas remote from industry varies between 1 and 2 ppm (Kaudy *et al.*, 1955). However, near sources of fluorine pollution in southern California, Kaudy *et al.* found elevated amounts of fluorine in citrus foliage, with as high as 211 ppm adjacent to a steel mill. Other industrial operations emitting fluorine involve aluminum, ceramics, phosphates, and miscellaneous chemicals.

Fluorine accumulates continually in leaves, particularly in the summer and fall months. Little increase was found after winter rains began (Kaudy *et al.*, 1955). The elevated fluorine concentrations found in citrus leaves were not associated with any visible damage to the trees. In fractionating leaves to determine the site of fluoride accumulation, Chang and Thompson (1965, 1966) found that navel oranges had the following decreasing order of subcellular distribution: cell wall, chloroplast, water soluble protein, and mitochondria. However, when allowance was made for cross contamination from chloroplasts and chloroplast fragments, it was concluded that the chloroplast fraction had the highest accumulation of fluoride.

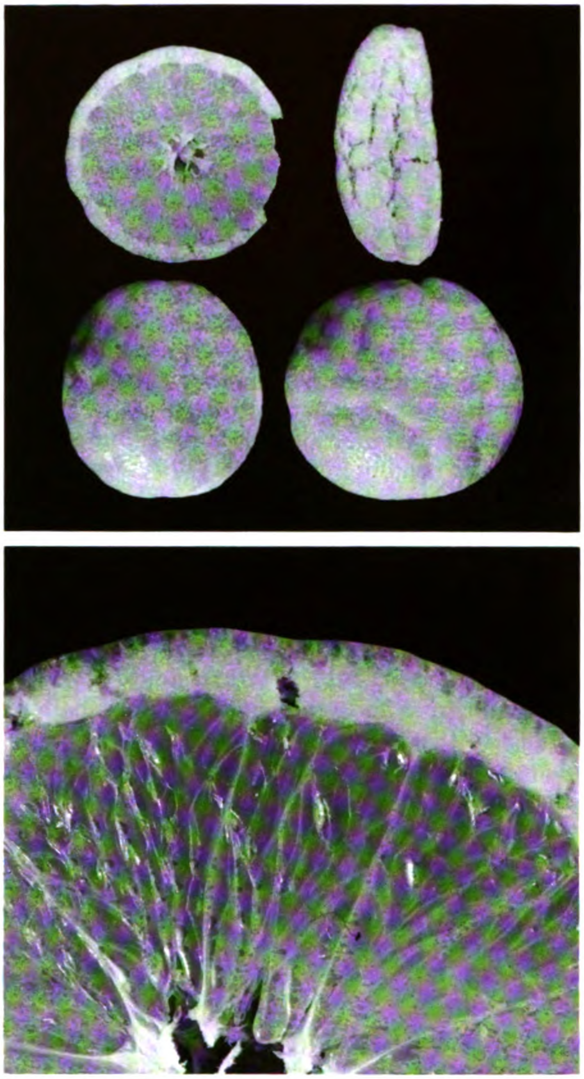

Fig. 2–21. Creasing in Valencia orange fruits. Note creases in whole fruit and broken albedo in the sections. In the whole fruit the creases occur over the broken area of the albedo.

In a study of varietal sensitivity, Brewer *et al.* (1960*a*) exposed seven varieties of young citrus trees to 10 to 12 parts per billion (ppb) of fluorine as hydrogen fluoride. The varieties studied were Lisbon and Eureka lemons, pink grapefruit (CES Redblush No. 3) and Marsh grapefruit, and Temple, navel and Valencia oranges. All varieties were sensitive to fluorine, but not equally so, nor did they respond in the same manner. Lemons developed the most chlorosis, oranges developed the least, and grapefruit were intermediate. On the other hand, oranges showed the most reduction in leaf size and gross growth. On an over-all evaluation, grapefruit was affected least.

Growth and vigor of navel orange trees were reduced by 2 to 3 ppb of hydrogen fluoride in the air, even though visible symptoms were lacking (Brewer *et al.*, 1960*b*). After five to seven months of continuous exposure to 3 to 5 ppb of hydrogen fluoride in the air, considerable chlorosis and some necrosis of mature leaf tips were found. The tip necrosis bore a resemblance to that produced by an excess of boron. Greater leaf drop was associated with hydrogen fluoride in the air. When citrus leaves are exposed to hydrogen fluoride in the air there is a gradual accumulation of fluorine in the leaves with hardly any translocation to stems and roots. With exposure to 2 to 3 ppb of hydrogen fluoride over a period of a year, the fluorine content in leaves, on a dry-weight basis, was found to increase to about 200 ppm. Fruit quality and yield were adversely affected by hydrogen fluoride, although the fruit accumulated very little fluorine. The most obvious effect was in a coarsening of the peel.

Wander and McBride (1956) sprayed hydrogen fluoride and fluosilicic acid on young grapefruit trees and produced a chlorosis pattern similar to that observed in the vicinity of phosphate rock-processing operations in Polk County, Florida.

Plant damage from ozone and other oxidizing materials in polluted air has been extensive in recent years in the Los Angeles basin. Although such damage has been readily evaluated on leafy vegetable crops, the effects are more subtle in the case of citrus. Spotting of leaves and fruit have been noted at certain times in oranges and grapefruit, but the extent of damage has not been determined. Freebairn and Taylor (1960) made a notable attempt to protect plants with a potassium ascorbate spray. A concentration of 0.001 molar potassium ascorbate had no effect on grapefruit, Cleopatra mandarin, and Sampson tangelo. The spray provided between 30 and 45 per cent protection at 0.01 molar, however, and at 0.05 molar the protection was 65 to 75 per cent. At the higher concentration, there was about 15 per cent leaf injury from the ascorbate itself.

Dugger, Koukol, and Palmer (1966) were not able to observe any visible symptoms when they exposed lemon seedlings to 0.25 ppm ozone. They did find that the ozone increased permeability and the rate of respiration of the cells in the leaves. Starch and total carbohydrates decreased in the leaves, although the reducing sugar level was higher.

Thompson *et al.* (1967) constructed plastic-covered enclosures over young orange and lemon trees in orchards and supplied the interiors with either ambient or filtered air. Removal of oxidants with activated charcoal filters resulted in increased CO_2 and water utilization, although the additional removal of ambient fluoride with limestone had no measurable effects.

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CHAPTER 3

The Mineral Nutrition of Citrus

HOMER D. CHAPMAN

Any comprehensive discussion of citrus nutrition should include sections dealing with the mechanism of nutrient absorption, the influence of one ion on the absorption of another, translocation and mobility of nutrients within the plant, the effects of light, temperature, and humidity on nutrition, the relation of soil to nutrition, internal physiology, and other topics. Two pertinent recent works are *Nutrition of Fruit Crops* edited by Childers (1966) and *Diagnostic Criteria for Plants and Soils* edited by Chapman (1966).

This chapter is confined to a discussion of the mineral composition of citrus, the effects of nutrient deficiencies and excesses, salinity, pH, aeration, and some consideration of both the causes of nutrient disorders and methods of control. A detailed discussion of fertilization is presented in Volume III.

THE MINERAL COMPOSITION OF CITRUS

Many analyses of the fruit and the various parts of citrus trees have been made. Among the early analyses, the most complete are found in publications by Thorpe (1868), Wolff (1871–80), Ricciardi (1880*a,* 1880*b*), Boschi (1895), and Oliveri and Guerrieri (1895).

Less complete, but more inclusive as regards the number of parts of the tree included, is an analysis of a nineteen-year-old Marsh Seedless grapefruit tree by Barnette *et al.* (1931). This analysis is especially valuable since the tree was mature and total weights of the various parts of the tree were recorded. Data from the analysis are reproduced in part in table 3–1.

Representative analyses of the important vegetative parts of citrus trees are assembled in table 3–2. In table 3–3, analyses of citrus fruits are brought together. Considering the number of factors affecting plant composition and their variability, the reported data are in fair agreement.

With respect to the quantities of ash constituents in various parts of the tree, it is evident that the leaves rank first. The more woody parts rank lowest; such determinations as have been made on bark and wood show that in ash content the bark is more nearly comparable with the leaves.

The percentage distribution of various elements in the ash is given in table 3–4. In all vegetative parts of citrus trees, calcium is the dominant constituent, comprising, in most parts, more than 34 per cent of the total weight of the ash. Next in abundance is potassium, comprising from 7 to 15 per cent. Lower, and all roughly of the same magnitude, are magnesium, phosphorus, sulphur, and silicon. Chlorine is the least abundant element. Sodium is variable. (Other constituents will be considered under trace elements.) In the fruit, potassium rather than calcium is the domi-

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Table 3–1

MINERAL COMPOSITION OF A NINETEEN-YEAR-OLD MARSH SEEDLESS GRAPEFRUIT TREE[*]

		Total Dry Weight			Mineral Composition[*]					
					Ca			Mg		
Tree Parts	Total Green Weight in Pounds	Pounds	Percentage of Total	Percentage in Dry Matter	Pounds	Percentage in Dry Matter	Percentage of Total	Pounds	Percentage in Dry Matter	Percentage of Total
Leaves	113.10	33.40	5.54	29.50	0.95	2.84	12.90	0.05	0.14	10.25
Immature fruit	76.20	16.60	2.75	21.75	0.03	0.21	0.41	0.02	0.11	4.17
Spring growth	12.60	5.10	0.84	40.40	0.09	1.79	1.23	0.01	0.11	2.08
Limbs up to ½ in. diameter	56.80	29.90	4.96	52.75	0.51	1.72	6.95	0.02	0.06	4.17
Limbs ½ to 2½ in. diameter	121.50	73.30	12.17	60.35	0.83	1.13	11.30	0.04	0.05	8.34
Old limbs	270.20	172.10	28.60	63.69	2.17	1.26	29.70	0.12	0.07	25.00
Trunk	109.40	63.40	10.51	57.92	0.85	1.35	11.58	0.05	0.08	10.25
Fibrous roots[†]	252.30	90.00	14.95	35.69	0.39	0.43	5.21	0.09	0.10	18.80
Large roots[†]	175.10	118.40	19.68	67.59	1.53	1.29	20.75	0.08	0.07	16.65
Total	1,187.20	602.20			7.35			0.48		
Weighted average				50.75		1.22			0.08	

	Mineral Composition[*]								
	K			P			N		
Tree Parts	Pounds	Percentage in Dry Matter	Percentage of Total	Pounds	Percentage in Dry Matter	Percentage of Total	Pounds	Percentage in Dry Matter	Percentage of Total
Leaves	0.82	2.46	19.40	0.05	0.14	8.32	0.81	2.41	17.80
Immature fruit	0.30	1.80	7.05	0.03	0.18	5.00	0.26	1.56	5.72
Spring growth	0.08	1.63	1.89	0.01	0.17	1.67	0.06	1.18	1.32
Limbs up to ½ in. diameter	0.20	0.66	4.71	0.03	0.09	5.00	0.20	0.67	4.41
Limbs ½ to 2½ in. diameter	0.34	0.46	8.04	0.04	0.05	6.67	0.30	0.41	6.61
Old limbs	0.69	0.40	16.26	0.07	0.04	11.65	0.66	0.38	14.52
Trunk	0.24	0.38	5.68	0.03	0.05	5.00	0.30	0.47	6.61
Fibrous roots[†]	1.02	1.13	23.60	0.19	0.21	31.70	0.92	1.02	20.24
Large roots[†]	0.54	0.46	12.78	0.15	0.13	25.50	1.03	0.87	22.66
Total	4.23			0.60			4.54		
Weighted average		0.70			0.10			0.75	

Source: After Barnette *et al.* (1931).

[*] Ca, Mg, K, and P figures reported as oxides in original paper.

[†] Calculated total from quantity of roots taken from one-eighth of soil area presumably occupied by roots to a depth of 4 feet.

Table 3–2

MINERAL COMPOSITION OF VEGETATIVE PARTS OF CITRUS TREES

Part of Tree	Kind of Citrus	Number of Samples	Mineral Composition in Percentages of Dry Weight											Source of Tree	Reported By
			Ash	Ca	Mg	K	Na	Fe	P	S	Si	Cl	N		
Leaves	Orange (navel)	3	15.65*	4.91	0.27	1.00	0.12	0.024	0.14	0.37	0.15	0.09	2.47	Calif.	Kelley and Cummins (1920)
Leaves	Orange (Valencia)	3	16.99*	5.81	0.26	0.71	0.10	0.019	0.16	0.33	0.19	0.04	2.24	Calif.	Kelley and Cummins (1920)
Leaves	Orange (Valencia)	1	13.72*	3.95	0.37	1.44	0.16	0.030	0.015	......	0.17	0.02	2.15	Calif.	Reed and Haas (1923*b*)
Leaves	Orange (Valencia)	4	10.33†	2.15	0.58	2.09	......	....	0.26	......	......	....	2.04	Florida	Blair (1910)
Leaves	Orange (Valencia)	1	....	4.48	0.54	......	......	0.015	0.24	......	......	....	2.31	Florida	Collison (1919)
Leaves	Grapefruit	1	....	2.20	0.14	2.46	......	....	0.14	......	......	....	2.41	Florida	Barnette *et al.* (1931)
Leaves	Orange (sweet)	1	6.35‡	2.86	0.22	0.79	0.01	0.035	0.13	0.08	0.07	0.04	1.71	Italy	Oliveri and Guerrieri (1895)
Leaves	Lemon	1	6.10‡	3.10	0.04	0.66	0.04	0.035	0.12	0.06	0.05	0.03	1.50	Italy	Oliveri and Guerrieri (1895)
Shoots	Orange (Valencia)	1	5.69†	1.58	0.17	0.55	0.08	0.049	0.14	......	0.06	0.01	0.79	Calif.	Reed and Haas (1923*b*)
Shoots	Orange (Valencia)	4	4.06†	0.99	0.36	0.95	......	....	0.18	......	......	....	1.00	Florida	Blair (1910)
Shoots	Orange	1	....	2.89	0.32	......	......	0.021	0.18	......	......	....	1.27	Florida	Collison (1919)
Limbs to ½ in. diameter	Grapefruit	1	....	1.72	0.60	0.66	......	....	0.09	......	......	....	0.67	Florida	Barnette *et al.* (1931)
Green wood	Orange (sweet)	1	6.20†	3.29	0.14	0.48	0.02	0.021	0.11	0.05	0.04	0.03	0.64	Italy	Oliveri and Guerrieri (1895)
Green wood	Lemon	1	6.17‡	3.59	0.05	0.48	0.01	0.014	0.12	0.05	0.04	0.02	0.67	Italy	Oliveri and Guerrieri (1895)
Trunk	Orange (Valencia)	1	2.08*	0.51	0.07	0.22	0.10	0.007	0.07	0.03	00.2	0.006	0.40	Calif.	Reed and Haas (1923*b*)
Trunk	Grapefruit	1	....	1.35	0.07	0.38	......	....	0.05	......	......	....	0.47	Florida	Barnette *et al.* (1931)
Dry wood	Orange (sweet)	1	6.17‡	3.22	0.11	0.54	0.03	0.014	0.10	0.04	0.09	0.01	0.28	Italy	Oliveri and Guerrieri (1895)
Dry wood	Lemon	1	6.48‡	3.40	0.05	0.61	0.03	0.014	0.14	0.08	0.04	0.01	0.41	Italy	Oliveri and Guerrieri (1895)
Roots	Orange (Valencia)	1	2.77*	0.70	0.05	0.28	0.09	0.021	....	0.06	0.06	0.016	0.82	Calif.	Reed and Haas (1923*b*)
Roots	Grapefruit	1	....	1.29	0.07	0.46	......	....	0.13	......	......	....	0.87	Florida	Barnette *et al.* (1931)

* Fully carbonated ash.
† Basis of reporting ash unknown.
‡ Total ash less ($C + CO_2$).

Table 3–3

MINERAL COMPOSITION OF CITRUS FRUITS

Kind of Fruit	Number of Samples	Percentage Composition on Fresh-Weight Basis: Ash	Ca	Mg	K	Na	Al_2O_3 + Fe_2O_3	Mn	P	S	Si	Cl	N	Source of Fruit	Reported By
Oranges and tangerines	10	0.915*	0.148	0.027	0.398	0.029	0.006	....	0.034	0.017	0.004	0.011	0.124	Florida	Pickel and Earle (1892)
Oranges	11	....	....	....	0.243	....	....	....	0.023	....	....	....	0.118	Florida	Blair (1909)
Oranges	1	0.787†	0.095	0.016	0.228	....	0.0006	Tr.	0.027	....	....	....	0.112	Florida	Bahrt and Hughes (1935)
Oranges	9	0.526§	0.070	0.014	0.175	0.008	0.004	0.0007	0.023	0.009	0.001	0.004	....	Calif.	Colby (1893)
Lemons	1	0.432‡	0.091	0.019	0.188	0.016	0.004	0.0014	0.028	0.008	0.002	0.004	....	Calif.	Colby (1893)
Lemons	1	0.784‡	0.201	0.026	0.196	0.017	0.016	0.0035	0.067	0.008	0.003	0.002	....	Calif.	Colby (1893)
Lemons	15 fruits	0.418§	0.073	0.013	0.138	0.008	0.009	....	0.024	0.007	0.003	0.001	0.163	Italy	Boschi (1895)
Lemons	15 fruits	0.540§	0.086	0.020	0.217	0.013	0.007	....	0.026	0.012	0.003	0.001	0.174	Italy	Boschi (1895)

* Sum of oxides and chlorine.

† Basis of reporting unknown.

‡ Percentage of pure ash (probably crude ash less C + CO_2 + sand).

§ Mineral matter minus CO_2.

nant base. The other elements are present in fruit ash in about the same proportion as in the ash of vegetative parts, phosphate being perhaps somewhat higher in the fruit ash.

The analysis of a nineteen-year-old grapefruit tree by Barnette *et al.* (1931) provided interesting information on the distribution of various elements in different parts of the tree (table 3–1). Most of the calcium and magnesium was found in the branches, the trunk, and the large roots; relatively more of the phosphate was present in the roots. Nitrogen and potassium were predominantly localized in the leaves and roots, with lesser amounts in the limbs and trunk. The analyses are assembled here to provide a generalized picture of amounts, proportions, and distribution of mineral constituents in citrus trees. They are suggestive of trends only. The proportions of total dry matter and ash in various parts of mature citrus trees often vary considerably. This is well brought out by comparing data for the nineteen-year-old grapefruit tree given above with data for a ten-year-old Valencia tree (Cameron and Appleman, 1934). For both trees, the entire weights of leaves, branches, trunk, and roots were recorded, and nitrogen analyses were made. Since data for the roots may be somewhat in error, results are given only for leaves, branches, and trunks (table 3–5). For the Valencia tree, it will be noted that foliage represented 28.35 per cent of the total dry weight and contained 59.39 per cent of the total nitrogen in the above-ground part of the tree, as compared with 8.85 per cent and 34.76 per cent, respectively, for the grapefruit tree.

The amounts and proportions of ash constituents in any part of a tree are profoundly influenced by age. This was strikingly shown in leaf analyses reported by Kelley and Cummins (1920), whose data are reproduced in part in table 3–6. As may be seen from the table, the leaves became progressively richer in ash and calcium with age. Increases in calcium were accompanied by decreases in potassium and phosphate.

Because of current interest in trace elements, miscellaneous analyses by various investigators are assembled in table 3–7. Of the various elements reported, iron is the most abundant, ranging roughly from 100 to 200 ppm in the dry leaf. Boron, aluminum, and fluorine range from 30 to 50 ppm. Manganese and zinc are less abundant, and copper is least abundant.

In addition to these analyses, data pub-

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Table 3–4

PERCENTAGE DISTRIBUTION OF ELEMENTS IN THE ASH OF FRUIT AND VARIOUS PARTS OF CITRUS TREES

Part of Tree	Percentage in Ash*								Reported By
	Ca	Mg	K	Na	P	S	Si	Cl	
Fruit (orange)	16.24	3.22	40.63	1.85	5.40	2.10	0.30	0.92	Colby (1893)†
Fruit (lemon)	15.89	3.78	40.31	2.54	4.87	2.32	0.50	0.28	Boschi (1895)†
Leaves (orange)	47.28	2.62	9.59	1.36	1.34	3.54	1.49	0.87	Kelley and Cummins (1920)‡
Leaves (orange)	44.97	3.41	12.40	0.25	2.04	1.17	1.19	0.66	Oliveri and Guerrieri (1895)†
Shoots (orange)	42.47	4.70	14.70	2.20	3.75	......	1.51	0.27	Reed and Haas (1923*b*)‡
Green wood (orange)	53.06	2.30	7.60	0.39	1.85	0.87	0.61	0.46	Oliveri and Guerrieri (1895)†
Trunk (orange)	33.96	4.43	14.44	6.94	4.67	1.87	1.26	0.40	Reed and Haas (1923*b*)‡
Old wood (orange)	52.13	1.74	8.84	0.51	1.67	0.64	1.45	0.25	Oliveri and Guerrieri (1895)†
Roots (orange)	39.46	2.71	15.87	3.75	......	3.38	3.17	0.90	Reed and Haas (1923*b*)‡
Roots (orange)	35.67	4.17	12.81	3.82	6.86	2.31	0.84	0.82	Wolff, vol. 11 (1871–80)†

* Original data reported as oxides, except for the element Cl.

† Data expressed as percentage of pure ash (probably crude ash less C + CO_2 + sand).

‡ Data expressed as percentage of total oxides plus Cl included in table.

Table 3–5

DISTRIBUTION OF DRY WEIGHT AND NITROGEN IN ABOVE-GROUND PARTS OF A NINETEEN-YEAR-OLD GRAPEFRUIT TREE AND A TEN-YEAR-OLD VALENCIA TREE

Part of Tree	Nineteen-year-old Grapefruit Tree*					Ten-year-old Valencia Tree†				
	Total Dry Weight in Grams	Total N in Grams	Percentage N	Percentage of Total Dry Weight	Percentage of Total N	Total Dry Weight in Grams	Total N in Grams	Percentage N	Percentage of Total Dry Weight	Percentage of Total N
Leaves	15,150	367.41	2.42	8.85	34.76	15,518	301.36	1.94	28.35	59.39
Twigs and branches	127,191	553.38	0.43	74.34	52.36	31,851	174.32	0.55	58.18	34.30
Trunk	28,757	136.08	0.47	16.81	12.88	7,373	31.78	0.43	13.47	6.26
Total	171,098	1,056.87	0.62	....	....	54,742	507.46	0.92	....	....

* Reported by Barnette *et al.* (1931).

† Reported by Cameron and Appleman (1934).

Table 3–6

COMPOSITION OF NAVEL ORANGE LEAVES AT DIFFERENT STAGES OF GROWTH

Age of Leaves	Percentage of Water	Percentage of Ash	Percentage of Dry Matter									
			Ca	Mg	K	Na	Fe	P	S	Si	Cl	N
One week	72.31	6.54•	1.36	0.26	1.30	0.08	0.018	0.36	0.25	0.06	0.02	3.01
Six weeks	70.81	9.20	2.62	0.30	0.95	0.04	0.02	0.21	0.27	0.04	0.01	2.45
Mature, 6 months to 2 years	60.98	16.95	5.63	0.37	0.96	0.08	0.02	0.13	0.36	0.14	0.05	2.39
Very old, 3 or more years (yellowed with age)	60.73	21.39	7.36	0.36	0.36	0.09	0.02	0.14	0.37	0.20	0.08	1.31

*Source:*Kelley and Cummins (1920); original data except for Cl and N reported as oxides.

• Completely carbonated ash.

Table 3–7

INORGANIC COMPOSITION OF CITRUS (TRACE ELEMENTS)

Part of Tree	Number of Analyses	Concentration (Ppm)	Reported By
		Aluminum	
Leaves (orange)	1	6.0–200.0 (dry wt.)	Chapman (1960)
Grapefruit (whole)	....	0.9 (green wt.)	Winter and Bird (1929)
Orange fruit (whole)	....	1.6 (green wt.)	Winter and Bird (1929)
Lemon fruit (whole)	....	1.5 (green wt.)	Winter and Bird (1929)
		Boron	
Leaves (orange)	Average of 9 samples	43.0 (dry wt.)	Kelley and Brown (1928)
Leaves (lemon)	Average of 5 samples	30.0 (dry wt.)	Kelley and Brown (1928)
Orange fruit (whole)	Average of 2 samples	2.6 (green wt.)	Scofield and Wilcox (1930)
		Copper	
Leaves (grapefruit)	2	6.9 (dry wt.)	Haas and Quayle (1935)
Leaves (lemon)	6	7.0 (dry wt.)	Haas and Quayle (1935)
Leaves (orange)	5	11.1 (dry wt.)	Haas and Quayle (1935)
Leaves (orange)	2	3.4 (dry wt.)	Chapman (1960)
Lemon fruit (whole)	6	3.7 (dry wt.)	Haas and Quayle (1935)
Orange fruit (whole)	1	4.4 (dry wt.)	Haas and Quayle (1935)
		Iron	
Leaves (orange)	4	104.0 (dry wt.)	Chapman (1960)
Leaves (grapefruit)	2	182.0 (dry wt.)	Haas and Quayle (1935)
Leaves (orange)	2	209.0 (dry wt.)	Haas and Quayle (1935)
Juice (orange)	1	2.8 (fresh wt.)	Peterson and Elvehjem (1928)
		Fluorine	
Leaves (orange)	1	50.0 (dry wt.)	Vanselow (Calif., unpub. data)
Orange fruit (whole)	2	0.8 (fresh wt.)	Vanselow (Calif., unpub. data)
		Manganese	
Leaves (orange)	3	24.0 (dry wt.)	Chapman (1960)
Leaves (orange)	3	27.0 (dry wt.)	Haas (1932*a*)
		Zinc	
Leaves (orange)	9	17.5 (dry wt.)	Thomas, Vanselow, and Stewart, 1935 (Calif., unpub. data)
Orange fruit (whole)	4	0.6 (fresh wt.)	Thomas *et al.*, 1935 (Calif., unpub. data)

Table 3–8

TRACE ELEMENT VARIATIONS IN CITRUS LEAVES

Element	Range in Dry Matter (Ppm) Low Range	Usual Range	High to Excess Range
Aluminum (Al)	6.0–20.0	6.0–30.0	40.0–200.0
Arsenic (As)	< 1.0	< 1.0	> 1.0–116.0
Barium (Ba)	5.0–20.0	5.0–49.0	50.0–250.0
Boron (B)	< 15.0–40.0	50.0–200.0	200.0–1,679.0
Bromine (Br)	....	200.0–1,000.0	1,000.0–18,000.0
Chromium (Cr)	Tr.–0.2	Tr.–0.5	> 1.0
Cobalt (Co)	< 0.4	Tr.–0.4	> 10.0
Copper (Cu)	< 4.0–5.0	5.0–15.0	15.0–> 20.0
Fluorine (F)	< 1.0–5.0	< 1.0–20.0	25.0–365.0
Gallium (Ga)	< 1.0	< 1.0–4.0	5.0–10.0
Indium (In)	< 1.0	< 1.0	1.0–2.0
Iron (Fe)	< 40.0–60.0	60.0–150.0	> 150.0
Lead (Pb)	< 0.5	< 1.0	2.0–20.0
Lithium (Li)	< 0.5	0.5–0.9	13.5–220.0
Manganese (Mn)	5.0–24.0	25.0–100.0	100.0–> 1,000.0
Molybdenum (Mo)	0.01–0.1	0.10–3.0	4.0–> 100.0
Nickel (Ni)	< 0.5	0.5–1.0	1.0–140.0
Silver (Ag)	< 0.05	0.1–0.5	> 1.0
Strontium (Sr)	30.0–90.0	40.0–90.0	200.0–1,000.0
Tin (Sn)	< 1.0	< 1.0–2.0	4.0–12.0
Titanium (Ti)	0.3–4.0	....	4.0–20.0
Vanadium (V)	1.5–3.0	....	....
Zinc (Zn)	4.0–24.0	25.0–100.0	110.0–200.0

Table 3–9

KINDS AND AMOUNTS OF MINOR ELEMENTS PRESENT IN ASH OF VARIOUS CITRUS JUICES AS INDICATED BY SPECTROGRAPHIC ESTIMATION

(In Parts Per Million)

Element	Seedling Orange	Blood Orange	Valencia Orange	Lue Gim Gong Orange	Marsh Seedless Grapefruit	Seedy Grapefruit	Tangerine
Ba	80–300	800–3,000	8–30	1–10	8–30	8–30	80–300
B	30–80	50–100	10–50	8–30	8–30	8–30	30–80
Cr	8–30	8–30	8–30	1–10	8–30	8–30	8–30
Cu	80–300	80–300	80–300	50–100	80–300	>1,000	800–3,000
Mn	80–300	80–300	50–100	30–80	80–300	80–300	300–800
Pb	N.D.•	8–30	N.D.	Trace	8–30	Trace	Trace
Ni	N.D.	Trace	N.D.	Trace	Trace	N.D.	Trace
Sn	8–30	80–300	Trace	N.D.	30–80	Trace	8–30
Sr	800–3,000	8–30	1–10	1–10	8–30	1–10	8–30
Ag	N.D.	Trace	Trace	Trace	1–10	Trace	Trace
Ti	N.D.	30–80	1–10	80–300	1–10	Trace	1–10
V	N.D.	N.D.	N.D.	N.D.	Trace	N.D.	N.D.
Zn	100–500	30–80	80–300	30–80	80–300	10–500	30–80
Zr	N.D.	8–30	Trace	Trace	1–10	8–30	8–30

Source: Roberts and Gaddum (1937).

• Not detected.

lished by Chapman (1960)—much of which was derived from unpublished information supplied by Vanselow—are shown in table 3–8. Roberts and Gaddum (1937) determined by spectrographic means the minor elements in various citrus fruit juices. Their results are recorded in table 3–9.

More recent data, showing the inorganic composition of leaves and fibrous roots of young Valencia orange trees fertilized with various amounts of nitrogen, potassium, and magnesium, have been published by Smith *et al.* (1954). These data are shown in table 3–10. In addition, Smith and Reuther (1953) published data relating whole fruit composition to variable fertilization with nitrogen, potassium, and phosphorus. These data are shown in table 3–11.

Some pertinent data on the variable composition of the rootlets of Valencia oranges (on rough lemon rootstock), showing marked differences in rootlet composition with soil depth, were published by Reuther, Embleton, and Jones (1958). These data from Florida orchard experiments are given in table 3–12.

Based on analyses recorded in the foregoing tables, calculations have been made showing the amounts of the various elements removed from an acre of soil by an average crop of oranges (20,000 pounds). These results (table 3–13) show that potassium, calcium, nitrogen, and phosphorus are the elements removed from the soil in the greatest quantity.

Table 3–10

MEAN MINERAL COMPOSITION (PORTION OF DRY MATTER) OF LEAVES AND FIBROUS ROOTS OF VALENCIA ORANGE TREES GROWN WITH DIFFERENT LEVELS OF NITROGEN, POTASSIUM, AND MAGNESIUM

	Mean Mineral Composition (Per Cent)											
	N		P		K		Ca		Mg		Na	
Treatment	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Low N	2.08	1.41	0.33	0.21	2.02	1.16	2.56	1.84	0.29	0.29	0.18	0.04
Intermediate N	2.33	1.46	0.16	0.13	1.11	0.98	3.24	1.92	0.27	0.40	0.13	0.09
High N	2.76	1.85	0.13	0.10	0.62	0.81	3.93	2.22	0.27	0.38	0.12	0.17
Low K	2.44	1.58	0.20	0.14	0.83	0.60	3.72	2.00	0.30	0.47	0.14	0.13
Intermediate K	2.39	1.57	0.22	0.15	1.12	0.82	3.43	1.97	0.27	0.40	0.14	0.12
High K	2.35	1.57	0.21	0.15	1.81	1.53	3.58	2.01	0.25	0.20	0.15	0.05
Low Mg	2.42	1.54	0.21	0.14	1.34	1.03	3.29	1.89	0.18	0.31	0.15	0.12
Intermediate Mg	2.38	1.54	0.22	0.15	1.29	0.94	3.28	2.08	0.26	0.35	0.14	0.09
High Mg	2.37	1.64	0.19	0.15	1.12	0.98	3.16	2.01	0.39	0.41	0.14	0.08
LSD at 5%*	0.08	0.06	0.023	0.008	0.122	0.08	3.15	0.26	0.014	0.038	0.016	0.015

	Mean Mineral Composition (Ppm)											
	Mn		Fe		Cu		Zn		B		Al	
Treatment	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Low N	46	214	65	1,720	15	94	75	422	163	25	143	586
Intermediate N	43	249	65	1,740	12	160	59	434	153	26	119	562
High N	42	308	69	1,890	13	218	46	465	122	25	104	653
Low K	42	256	67	1,730	13	152	58	462	163	25	116	575
Intermediate K	44	243	65	1,700	13	149	60	479	148	25	123	590
High K	44	271	67	1,920	14	172	62	479	127	26	127	636
Low Mg	41	263	68	1,650	15	154	56	546	148	25	130	616
Intermediate Mg	41	253	69	1,860	13	167	58	449	149	25	123	576
High Mg	48	254	63	1,840	12	151	66	426	141	26	113	609
LSD at 5%*	3.2	17.2	N.S.	N.S.	1.4	24.8	6.1	54.0	9.9	N.S.	12.2	72.0

Source: Smith *et al.* (1954).

[*] Least significant difference between means at odds of nineteen to one. Nonsignificance of differences between means is indicated by N.S.

Table 3–11

POUNDS OF INDIVIDUAL ELEMENTS IN 1,000 POUNDS OF FRESH CITRUS FRUITS IN RELATION TO VARIETY, ROOTSTOCK, AND FERTILIZATION

Fertilizer	N	P	K	Ca	Mg	Mn	Fe	Cu	Zn	B	Water (Per Cent)
					Valencia Orange on Rough Lemon						
0.9 lb N per tree	1.27	0.21	1.90	0.30	0.17	0.0013	0.005	0.0007	0.0024	0.0025	82.4
1.8 lb N per tree	1.27	0.20	1.97	0.37	0.16	0.0011	0.004	0.0007	0.0021	0.0024	82.0
3.6 lb N per tree	1.32	0.19	1.80	0.39	0.19	0.0014	0.005	0.0008	0.0027	0.0028	81.8
0.3 lb K_2O per tree	1.41	0.22	1.39	0.45	0.19	0.0013	0.005	0.0008	0.0025	0.0031	80.4
1.8 lb K_2O per tree	1.23	0.19	2.00	0.34	0.18	0.0011	0.004	0.0006	0.0024	0.0025	82.6
3.6 lb K_2O per tree	1.22	0.19	2.15	0.28	0.17	0.0013	0.005	0.0007	0.0023	0.0022	83.2
					Pineapple Orange on Rough Lemon						
0.0 lb P_2O_5 per tree	1.19	0.19	2.30	0.77	0.25	0.0014	0.005	0.0007	0.0015	0.0030	82.4
0.6 lb P_2O_5 per tree	1.18	0.19	2.33	0.76	0.24	0.0014	0.006	0.0007	0.0017	0.0028	82.6
1.8 lb P_2O_5 per tree	1.13	0.18	2.39	0.91	0.26	0.0013	0.005	0.0007	0.0018	0.0031	81.9
4.8 lb P_2O_5 per tree	1.23	0.19	2.52	0.90	0.24	0.0012	0.006	0.0007	0.0012	0.0032	82.0
					Marsh Grapefruit on Rough Lemon						
Various	1.06	0.13	2.02	0.41	0.11	0.0004	0.003	....	....	....	90.0
					Valencia Orange on Rough Lemon						
Various	1.23	0.23	2.77	0.69	0.13	0.0004	0.002	0.0004	0.0013	....	83.9
					Valencia Orange on Sour Orange						
Various	1.57	0.25	2.54	0.70	0.12	0.0008	0.002	0.0007	0.0010	....	85.5
					Pineapple Orange on Rough Lemon						
Various	1.52	0.26	2.81	0.60	0.16	0.0009	0.003	0.0004	0.0013	....	84.6
					World Citrus[*]						
	1.17	0.27	2.25	1.05	0.20	0.0007	0.003	0.0004	0.0006	0.0026	......

Source: Smith and Reuther (1953); their original table showed pounds of elements in 2,000 pounds of fruit.

[*] Calculated from averaged data by Chapman and Kelley (1943); mostly from 1892–95 data.

Table 3–12

EFFECT OF SOIL DEPTH ON COMPOSITION OF ROOTLETS OF VALENCIA ORANGE TREES ON ROUGH LEMON STOCK

Fertilizer Treatment[*]	Depth of Rootlets in Soil (Inches)	Concentration in Dry Matter[†]						
		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (Ppm)	Zn (Ppm)
$N_1K_1M_1$	0–10	1.22	0.20	0.46	0.37	0.64	241	1,810
	11–30	0.89	0.14	1.05	0.22	0.41	59	585
	31–60	0.80	0.14	0.94	0.16	0.32	16	161
	61–108	1.06	0.20	0.81	0.15	0.44	29	369
$N_3K_1Mg_1$	0–10	1.46	0.15	0.37	0.34	0.47	296	2,448
	11–30	0.97	0.09	0.61	0.19	0.41	112	1,545
	31–60	1.15	0.10	0.73	0.13	0.25	37	472
	61–108	0.97	0.13	0.54	0.20	0.47	75	257
$N_3K_3Mg_1$	0–10	2.02	0.18	2.20	0.66	0.41	250	2,108
	11–30	1.42	0.12	2.93	0.32	0.19	66	508
	31–60	1.25	0.10	3.15	0.22	0.11	30	255
	61–108	1.32	0.11	3.51	0.20	0.13	61	191
$N_3K_3Mg_3$	0–10	1.55	0.16	2.06	0.39	0.44	248	1,721
	11–30	1.12	0.09	2.46	0.24	0.29	80	648
	31–60	1.14	0.09	3.34	0.19	0.15	81	408
	61–108	1.43	0.12	5.05	0.20	0.26	32	675

Source: Reuther *et al.* (1958).

[*] The symbol N_1 indicates a low level of nitrogen fertilization, and N_3 a high level, etc.

[†] Corrected to SiO_2-free basis by deducting weight of acid-insoluble ash from dry weight of sample. Each value is the mean of four determinations for each of four plots.

Table 3–13

POUNDS PER ACRE OF MINERAL CONSTITUENTS REMOVED BY ORANGES (20,000 POUNDS OF FRESH FRUIT); BASED ON ANALYSES SHOWN IN TABLES 3–3 AND 3–7

Constituents	Pounds Removed by 20,000 Pounds of Fresh Fruit
Ca	20.9
Mg	3.8
K	51.1
Na	3.6
P	5.3
S	2.6
N	23.6
Si	0.560
Fe	0.056
B	0.052
Al	0.032
F	0.016
Zn	0.013
Cu	0.008

ALUMINUM (Al)

There is no evidence as yet that aluminum is essential for the growth of citrus or other higher plants. Much work, as is well known, has been done on aluminum toxicity.

In water-culture experiments with lemon cuttings, Liebig, Vanselow, and Chapman (1942) produced healthy plants with aluminum at concentrations as low as 0.005 ppm in the culture solution. Leaves from these plants showed 28 ppm in the dry matter; whole stems, 5.6 ppm; root wood, 4.5 ppm; root bark, 92 ppm; and fine roots, 280 ppm. Comparable plants in solutions containing 0.5 ppm of aluminum showed the following concentrations in the dry matter: leaves, 22; stems, 4.5; root wood, 9.2; root bark, 700; and fine roots, 1,000 ppm. Aluminum at rates of 0.1, 0.5, 2.5, and 5 ppm in the culture solution offset the toxic effect of copper when it was present in concentrations of 0.1 ppm, but at 0.02 ppm of copper, as little as 0.1 ppm of aluminum somewhat decreased top growth, and 2.5 and 5 ppm of aluminum exerted a substantial depressing effect on top growth but did not affect root growth. In fact, levels of 2.5 and 5 ppm of aluminum tended to stimulate root growth. The reasons for this behavior were not ascertained. Blevins and Massey (1959) and Hiatt, Amos, and Massey (1963), working with wheat and excised wheat roots, respectively, confirmed that aluminum can decrease copper uptake. The latter investigators believe that aluminum competes with copper for binding sites on plant roots. They also allude to further research which suggests that aluminum can markedly reduce manganese uptake by tobacco. Rees and Sidrak (1961) found that aluminum reduced manganese toxicity in *Atriplex hastata* var. *deltoidea,* but not in spinach and barley. Whatever the mechanism involved, it is probable that low concentrations of aluminum in the 0.1- to 1-ppm range may (under conditions of excess copper, manganese, and probably other metal ions) exert a beneficial effect on plant nutrition.

The toxicity of aluminum to plants is well known. In a recent summary of this subject, Pratt (1966) reviewed the results of controlled culture work by many investigators with quite a range of plant types. He noted, in general, growth reduction from nutrient concentrations ranging from 0.1 to over 5 ppm, depending on plant and nutrient conditions.

Because of its relatively high content in soils and probable low plant requirement (should aluminum prove indispensable), it seems unlikely that lack of aluminum will ever become a problem with citrus or other plants. On the other hand, aluminum salt or chelate additions to soils might prove beneficial in situations where copper or manganese toxicity is a problem. Aluminum may, in fact, be a natural factor in soils in offsetting to some extent the toxicity of copper, manganese, and other constituents made soluble under acid soil conditions. It may also pose toxicity problems.

Aluminum does not appear to be transported significantly to citrus tops and does not produce specific toxicity symptoms. The seat of toxicity appears to be in the roots, exerting simply a depressing effect on top growth. Aluminum's effect on copper uptake and perhaps manganese and other essential nutrients might, under some conditions, produce deficiencies of these elements.

ARSENIC (As)

Special interest attaches to arsenic in relation to citrus nutrition because of early findings that arsenical sprays reduce fruit acid and thus make for earlier maturity. While there is no evidence that arsenic is essential for citrus, it is toxic to plants. In so-called "normal" soils, arsenic ranges only from <1 to about 50 ppm (according to a survey of the literature made by Swaine, 1955), but in some soils, where it has been used as a pesticide or herbicide, much higher amounts are found.

Arsenic is still used some in snail baits and also in sprays to hasten citrus fruit maturity. Some well and spring waters contain abnormal amounts of arsenic, and Fergus (1955) found several soils near Brisbane, Australia, where the natural levels of arsenic were sufficient to cause disorders in bananas. These soils contained from 100 to 200 ppm of total arsenic. The source of the arsenic was traced to veined quartzite rock outcrops and fragments, apparently coming from mispickel in the rock veins.

Liebig, Bradford, and Vanselow (1959) reported that lemon cuttings grown in solution cultures were injured by 5 ppm of arsenite arsenic and 10 ppm of arsenate form. Root tips showed injury first, and leaves wilted as the injury progressed; at 10 ppm of arsenic as arsenite, the lemon cuttings died after eleven days. Arsenate was less toxic than arsenite at equivalent concentrations. The roots of plants grown at the 10-ppm arsenic level gradually became stubby, and root analyses showed up to 1,200 ppm in the dry matter of plants grown in solutions containing 10 ppm, as compared to 0.15 ppm in solutions to which no arsenic was added. A little root toxicity eventually showed up at 2 ppm of arsenite and 5 ppm of arsenate arsenic. Tender, new roots were more susceptible to injury than older roots. Because of root injury, arsenic does not appear to accumulate appreciably in the tops of plants, though Liebig *et al.* (1959) found up to 11 ppm of arsenic in the old leaves of affected plants, as compared to 0.10 to 0.15 ppm in comparable leaves from arsenic-free cultures. Old leaves contained more arsenic than young leaves, and (from Liebig's experiments) values for total arsenic in excess of 1 ppm in old, lower leaves of citrus suggest the need for further exploratory work to determine the possibility of toxicity.

An interesting observation in Liebig's experiment was that 1 ppm of arsenic produced a stimulating effect on roots but not on top growth.

The only abnormal leaf symptom to develop in lemon cuttings receiving excess arsenic was vein clearing—"vein chlorosis" or "yellow vein." This condition results from root rotting, breaks in the vascular system, or (particularly in lemons) from nitrogen deficiency. Impaired root activity and absorption of nutrients was probably the cause of this condition in these experiments with excess arsenic.

Working with Pineapple orange seedlings, Rasmussen and Henry (1964) found that as little as 0.1 ppm of arsenic as potassium arsenate in the nutrient solution at pH 5 to 6 reduced growth significantly, whereas 0.25 ppm reduced growth to about one-half of the controls. These levels were associated with about 0.4 to 0.5 ppm and 0.5 to 0.6 ppm, respectively, in leaves and 40 to 50 ppm and 50 to 70 ppm in roots. Most seedlings died after a few weeks at 4 ppm. In sand cultures, about 1 ppm in the substrate reduced growth about the same as 0.1 ppm in solution cultures.

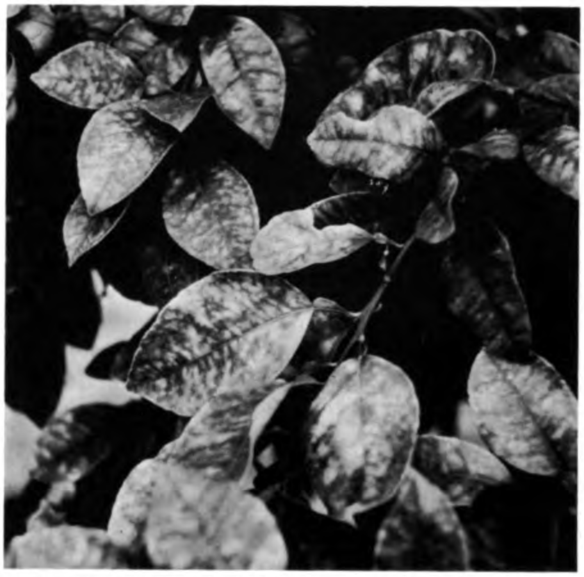

Fig. 3–1. Grapefruit leaves mottled from effects of arsenic sprays. (Photo courtesy of H. J. Reitz, Florida Agricultural Experiment Station, Lake Alfred, Florida.)

A type of symptom seen on grapefruit in Florida, resulting from arsenical sprays, is shown in figure 3–1.

It has long been known that arsenical sprays or dusts will decrease the acidity of citrus fruit. This fact was first reported in the *Florida State Horticultural Society Proceedings* of 1894 (vol. 7, p. 81), and subsequent studies in Florida, California, South Africa, and Japan confirmed these early findings. Miller, Bassett, and Yothers (1933) reviewed the early work on this subject and made a thorough study of the effects of lead arsenate sprays on the arsenic content of leaves, wood, fruit, and blossoms; they also studied its persistence on or in the tree, in the soil, and its effect on fruit composition, foliage, and fruit metabolism.

They found that arsenical sprays increased respiration rate in leaves and that catalase activity was increased by dilute arsenical sprays, but markedly decreased where more concentrated sprays were used. These metabolic changes were related to the fruit acid decreases. Fruit acid in oranges was decreased in unsprayed trees from 1.5 per cent to 0.5 per cent where the arsenous oxide of the leaves showed values of 0.1 mgm in 10 gm of fresh leaves. When arsenic reached concentrations of 1 mgm of arsenous oxide in 10 gm of fresh leaves, respiration increases ranged from 50 to 75 per cent, and leaves usually dropped. Catalase activity was increased when arsenous oxide was around 0.01 mgm per 10 gm of fresh leaves, but almost stopped when the arsenous-oxide levels reached 1 mgm. Very little arsenic found its way into the fruit juice. These authors reported that even after seventeen sprayings at 5 gallons per spray, only 0.16 mgm of arsenous oxide was found in a liter of juice.

Repeated lead arsenate sprays applied to the soil under citrus trees led to the accumulation of 2,000 ppm of arsenous oxide in the first 2 inches of soil, and 6 ppm in the 8- to 10-inch depth; comparable untreated horizons showed only 0.04 ppm. However, there was very little arsenic in the leaves of trees growing on the arsenic-contaminated soil, and there were no effects on growth, foliage color, fruit acid, and solids. This work was carried out on a Norfolk sand in an area where the annual rainfall is about 55 inches.

Roy (1943) and Gardner (1943) reported a sand-culture study with Duncan grapefruit trees on sour orange rootstock. Increasing amounts of sodium arsenate were added to the sand culture at rates of 10, 50, 200, and 800 ppm of arsenic. At 800 ppm, the trees began to defoliate after two months, and the few leaves remaining showed arsenic levels of 116 ppm. Leaves from trees growing in cultures receiving 200 ppm of arsenic contained 83 ppm of arsenic. The fruits from these trees were yellowish-green in color and showed brown stains in the albedo similar to the effects produced by boron deficiency. A theory was advanced that boron may function in the plant as part of an enzyme molecule and that arsenic interferes with this enzyme, producing the same effects as lack of boron.

BORON (B)

Boron Deficiency

The first researcher to produce and describe symptoms of boron deficiency on citrus was Haas (1929*b*, 1930). Later, Haas and Klotz (1931) described some of the anatomical and chemical changes produced in young citrus plants when this element is lacking. Morris (1937, 1938), in Rhodesia, South Africa, was the first to diagnose boron deficiency of citrus in the field and describe the symptoms.

The most conspicuous symptom produced under greenhouse conditions, as described by Haas (1929*b*), was a spectacular enlargement, splitting, and corking of leaf veins (see fig. 3–2, *D*). However, in the field, Morris (1938) found none of these symptoms, but described a fruit condition to which he gave the name "hard fruit." Subsequently, Chapman and Kelley (1943), in a fertilizer experiment begun in 1934 with navel orange trees in 55-gallon containers (using a granite-derived soil), produced boron deficiency and found both the "hard fruit" condition described by Morris (1938) and some of the vein splitting of leaves and bark splitting and gumming described by Haas (1929*b*), together with the abortion of young growth and dieback described by both investigators. The conditions were cured by either foliar sprays or soil additions of boric acid. The boron content of leaves (Chapman and Kelley, 1943) ranged from 5 to 15 ppm in the dry matter. Subsequent to Morris's findings, Camp (1939) noted symptoms in trees in Florida akin to those reported from Rhodesia; later still, Smith and Reuther (1950*a*) described symptoms on field trees in Florida, noting all of the fruit and foliage symptoms previously described. The boron content of the foliage showing strong deficiency symptoms averaged 14 ppm. These trees responded to sprays containing one pound of borax to 100 gallons of water, as well as to soil applications of three ounces of borax per tree. Meanwhile, Roy (1943), in Florida, and Haas (1945*a*), in California, published the results of controlled culture experiments, confirming the range of symptoms as described by earlier investigators and supplying further data on the boron content of deficient citrus plants. In further work, Smith and Reuther (1951) determined the effects on citrus tree size, foliar symptoms, leaf analysis, and vitamin C in fruit from applications of 0.01, 0.5, and 2 ppm of boron to sand cultures. At concentrations between those producing near deficiency and slight excess, no special effects on growth, fruiting, or mineral content were found. In Japan, Ishihara *et al.* (1965) found soil applications of borax increased fruit size and the proportion of well developed seed in satsuma. As a result of all this work, a fairly complete understanding of the foliage, growth, and fruit conditions produced by moderate to acute boron deficiency is at hand, including information on the range of boron levels in leaves which show typical hunger symptoms.

Table 3–14 presents a tabulation of the foliar, growth, and fruit characteristics which may be produced when boron is lacking, together with leaf analysis values associated with boron deficiency. Also shown for each abnormality are other known conditions which can produce identical or somewhat similar symptoms.

It is immediately apparent that no *one* of the visual foliar, growth, or fruit symptoms is of itself a sure sign of boron deficiency; thus, leaf curling, lack of luster, dieback, vein enlargement, splitting and gumming of twigs and branches, and the fruit abnormalities listed are common to other nutritional disorders, or some of these could be damage effects from insects, insecticides, hail, ice, and other causes.

When observations of visual effects are supplemented by leaf analyses, however, diagnosis can be almost certain. In other words, trees showing the complete syndrome of visual effects, together with leaf values showing 5 to 15 ppm of total boron, are almost certainly deficient in boron.

DeVilliers and Beyers (1961) got boron responses in citrus trees which showed up to 50 ppm of total boron in the dry matter of leaves, and they found evidence that water-soluble boron in leaves may be a better index of adequacy or inadequacy than total boron. Values of less than 15 ppm of water-soluble boron in the dry matter would be considered low; values well above this would be considered sufficient.

Boron deficiency effects are shown in figures 3–2 and 3–3. The fruit symptom on citrus most indicative of boron deficiency is the presence of a darkish, irregular ring or various localized darkish-colored spots in the white albedo of young orange fruits. The only other conditions known to produce such symptoms are (1) excess arsenic (likely to be encountered only where arsenical sprays are used), and (2) a virus disease reported in Sicily. The fruit symptoms of boron deficiency (or arsenic toxicity) are shown in figure 3–2. Where such symptoms are encountered and if leaf analysis shows total boron in the dry matter in the range of 3 to 15 ppm, it is almost certain that boron is lacking. Roy (1943) has shown that excess arsenic (which produces similar symptoms) does not impede boron uptake, and leaves from such plants will show normal boron levels (i.e., 30 ppm or more of boron.)

Early Symptoms of Boron Deficiency.—As with many other deficiencies, no information is available that would afford a positive diagnosis of the early stages of boron deficiency. However, low yields, abnormal abortion of young fruit, dieback of new growth, young fruit with albedo discoloration, and a foliage content of 25 ppm of boron or less would be suggestive and warrant trial applications of borax sprays or soil applications.

Causes of Boron Deficiency.—Boron deficiency commonly occurs in soils basically low in boron content (such as those derived from igneous rocks), or where crop removal has depleted the supply or liming has decreased boron availability.

Unfortunately, insufficient soil analysis data from soil areas where boron deficiency of citrus has been found in the field are at hand to be of use; but the boron content of the granitic soil in which boron deficiency ultimately developed in the author's oil-drum experiment (Chapman and Kelley, 1943), showed, at the outset, 10 ppm of *total* boron in the soil. Many soils derived from igneous-type rocks run much lower, as has been shown by Whetstone, Robinson, and Byers (1942), and the acid-soluble portion (which is probably the only portion available to plants) runs less than half the total, on the average. It has been suggested by Whetstone *et al.* (1942) that soils containing less than 10 ppm of boron (as determined by digestion in 85 per cent phosphoric acid followed by distillation with methyl alcohol) are suspect with regard to boron adequacy. Unless the fertilizer program or irrigation water adds enough to meet plant needs, such soils are likely to become boron-deficient.

Whetstone *et al.* (1942) analyzed some 300 soils representative of most of the major soil types in the United States and got values for total boron ranging from 4 to 133 ppm. The lowest values generally were obtained on soils derived from basic igneous rocks, such as granite, gneiss, and schist. Soils derived from shales, limestone, and sedimentary rocks generally are higher in boron.

(Text resumes on page 143)

Table 3–14

PRINCIPAL BORON DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Boron Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Translucent, water-soaked flecks or spots in young leaves with more or less deformation of such leaves.	1. Insects and some insecticides can cause somewhat similar effects—i.e., translucent spots and deformation of leaves.
2. Yellowing along midrib and lateral veins of mature and aging leaves.	2. Nitrogen and phosphorus deficiencies, old age, root rotting, or damage to vascular system will often cause vein yellowing in leaves.
3. Enlargement of midrib and lateral veins, splitting and corking (see figs. 3–2 and 3–3) of same on upper surface; comes on to a greater or lesser degree as leaves get old; not always seen on field trees, but a prominent symptom on plants grown under controlled conditions in greenhouse.	3. Magnesium, nitrogen, and potassium deficiencies, respectively, can cause splitting and corking of leaf veins. Some virus diseases also can cause vein splitting.
4. Old leaves are often thickened, leathery, deformed, lusterless, curled, and buckled.	4. Old age, thrips and aphid damage, potassium deficiency, and other factors cause leaves to be thickened, lusterless, curled, and buckled.
5. Death and abortion of very young new shoots, dieback of twig ends, excessive initiation, followed by death and abscission, of lateral shoots, multiple bud formation, and rosetting (see fig. 3–2).	5. Salt injury and calcium, zinc, and other deficiencies can bring on some of these effects.
6. Dieback of twigs and shoots (see fig. 3–3).	6. Copper, potassium, zinc, and other deficiencies in acute stages and salt excesses will cause dieback. There are many other causes of twig dieback.
7. Gum formation, cracking, and splitting of internodes of stems, branches, and trunk (see fig. 3–3).	7. Copper deficiency and excess, red scale infestation, or gummosis due to Phytophthora.
8. Normal to excessive blossoming in the spring.	8. Sulfur deficiency will sometimes cause excessive blossoming; also, quick decline and other factors can cause excessive blossoming.
9. Trees may show wilt before soil has reached wilting point.	9. Wilt and droopy appearance can be caused by root disease, root rotting, and, of course, lack of soil moisture.
Fruiting and Fruit Characteristics	
1. Gum pockets and grayish to brownish discoloration in albedo of young and mature fruits (see fig. 3–2).	1. Arsenic sprays and excess arsenic absorption from nutrient medium can cause some discolorations in albedo; a virus disease (impietratura) may produce similar effects.
2. Sometimes gum pockets in core and fleshy part of fruit (young and mature).	2. Copper deficiency often produces brown gum pockets in segments next to central pith of fruit.
3. Misshapen, hard fruit with thickened rinds.	3. Misshapen fruits with thick rinds occur when phosphate is lacking, but fruit is spongy instead of hard. Virus diseases can also produce misshapen fruit.
4. Rind of young fruit may show drying and darkening in patches where albedo gumming breaks through to surface (see fig. 3–2).	4. Rind breakdown and discoloration may be caused by other disorders such as insects, insecticides, hail, frost, and other factors.
5. Shriveled and/or darkened seed coat or lack of seed.	5. Other factors can also cause these effects; e.g., stubborn virus disease.
6. Fruit low in sugar.	6. Phosphorus excess produces low-acid, low-sugar fruit.
7. Juice content often low and fruit has dry interiors.	7. Sulfur deficiency can cause similar effects on fruit.
8. Excessive fall of young fruit, resulting in poor yield.	8. Phosphorus deficiency results in excessive drop of young fruit and low yields.
Tissue Analysis Values	
1. Total boron in mature leaves from trees deficient in boron ranges from 3 to ± 25 ppm in dry matter, whereas trees showing no symptoms of either boron deficiency or excess may range from 30 to ± 200 ppm. Scofield and Wilcox (1931) report up to 285 ppm boron in leaves showing no injury symptoms.	1. Other than boron deficiency, no conditions are known which result in boron levels in the low part of the 3- to 25-ppm range.

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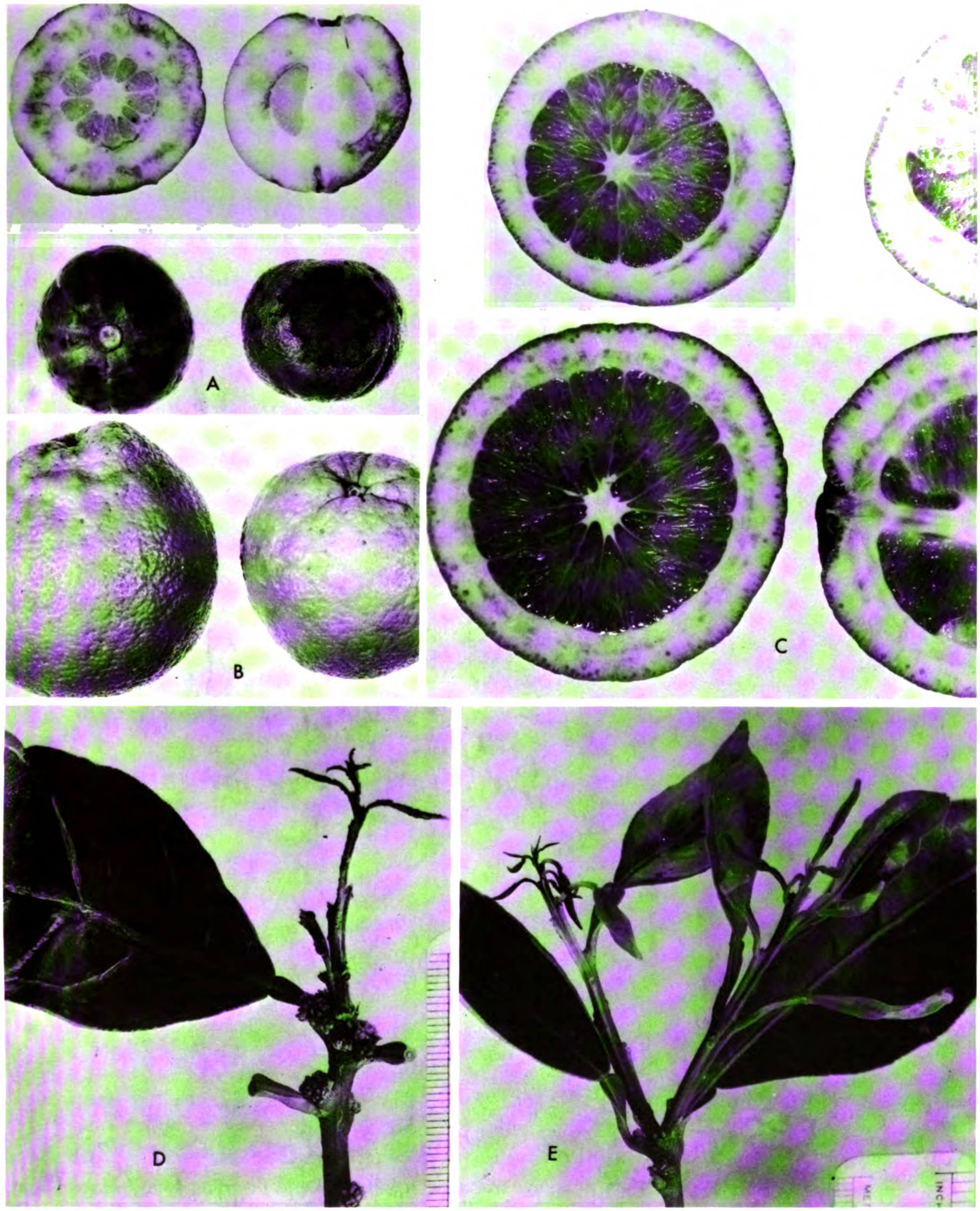

Fig. 3–2. Boron deficiency effects. *A*, discolorations in white albedo of young fruits and rind breakdown; *B*, misshapen, small, hard fruits; *C*, discoloration of white albedo in mature fruits; *D*, death and abortion of young terminal growth, multiple buds, and corking of veins; *E*, misshapen leaves, weak new growth, and death of new young terminals.

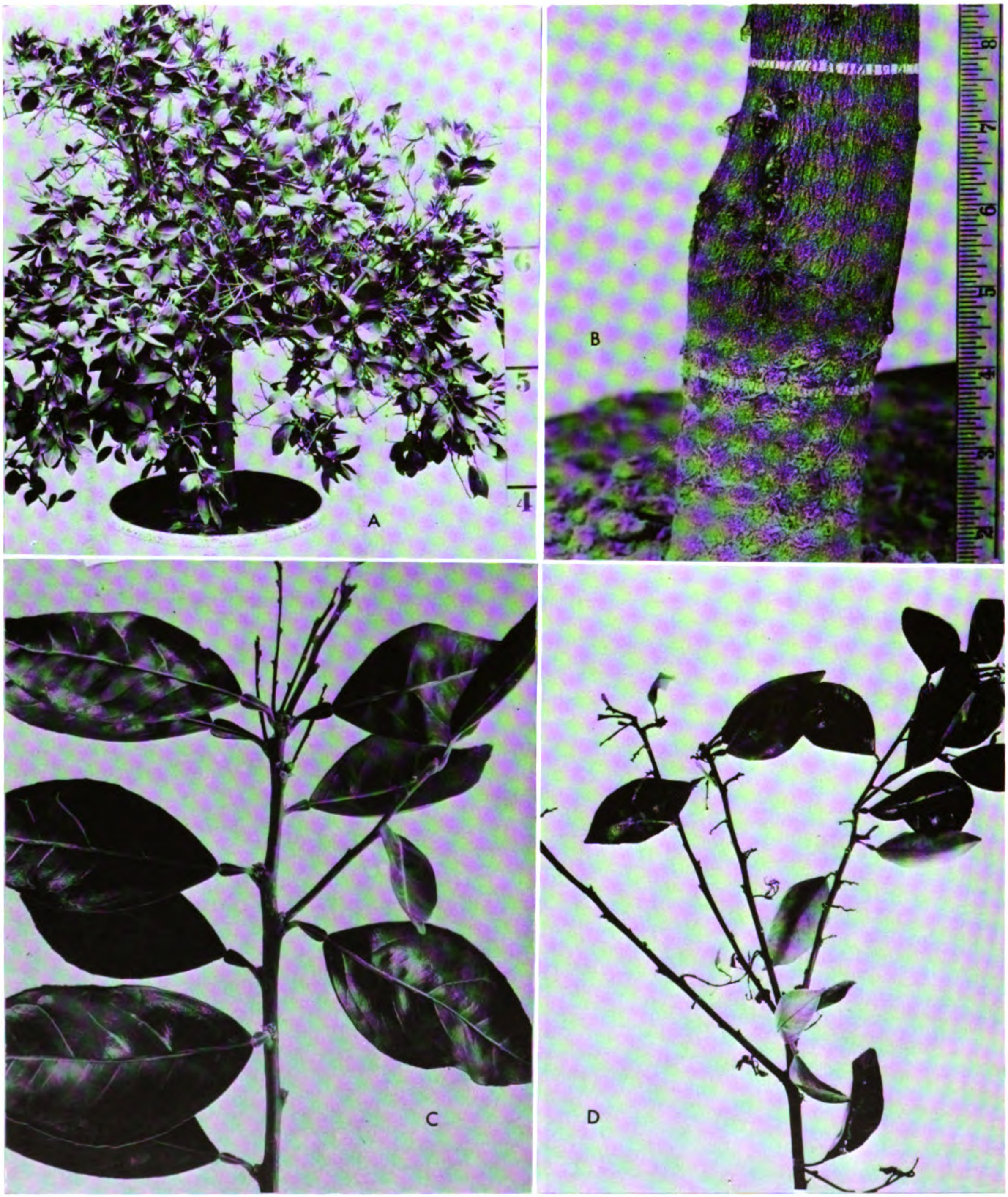

Fig. 3–3. Boron deficiency effects. *A*, navel orange tree showing dieback and dwarfing from lack of boron. At this stage, the leaf and fruit symptoms shown in this illustration and in fig. 3–2 can be seen at appropriate stages of growth. *B*, gum exudation on trunk from lack of boron. This effect is similar to that produced by Phytophthora fungus infections. *C*, old leaves showing split and corky midribs and veins and dieback of terminal growth; other malnutrition conditions can produce this same leaf condition. *D*, abortion of young terminal growth and excessive defoliation of old leaves commonly seen in spring of year at time of new growth.

Swaine (1955) reported values for total boron in soils ranging from 2 to 100 ppm.

From the evidence available, boron deficiency is to be looked for in the following kinds of soils: (1) those naturally low in boron, e.g., those derived from igneous and metamorphic or sandstone rocks; (2) those which are acid regardless of parent material, where the more soluble fractions have been leached out; (3) those which, other factors being equal, are low in colloid content; (4) those where laterization has taken place, i.e., soils which become progressively lower in silica compared with iron and aluminum; (5) acid muck and peat soils; and (6) alkaline soils which, while commonly containing ample boron, are low in available supply. From this it is understandable why liming of acid soils has often induced boron deficiency; conversely, the acidification and leaching of alkaline soils will accelerate the outgo of boron from soils and can bring on boron deficiency. Bingham, Martin, and Chastain (1958) have noted that high phosphate decreases boron absorption.

In addition to soil factors, boron deficiency in citrus is more likely to occur with citrus varieties grown on sour orange rootstock, since it appears that the feeding power or permeability of cells for boron in sour orange is less than that of other common rootstocks used for citrus. (This subject will be reviewed in greater detail in the discussion below of boron excess.)

Control of Boron Deficiency.—Correction of boron deficiency is a comparatively simple matter. Nutritional sprays, using either boric acid or borax at rates of one pound per 100 gallons of water, or soil applications of either of these compounds at rates of ¼ to ½ pound per tree, give control. Because of the narrow range between deficiency and toxicity, low application rates are definitely safer. Smith and Reuther (1950*a*) reported that three ounces of borax broadcast per tree produced correction on the sandy soils of Florida. Morris (1938), in South Africa, used rates varying from 1 to 35 ounces per tree. The heavier application produced no injury, but the soil was heavy and probably alkaline, and under these conditions the danger of injury is less.

Boron Excess

Boron excess produces a characteristic apical leaf mottling which is easily recognizable when fully developed and can usually be distinguished by leaf and soil analyses from other conditions producing similar symptoms. The principal effects of boron excess are outlined in table 3–15 (see fig. 3–4 for illustrations).

Excess sulfate produces a very similar type of apical leaf mottling, and somewhat similar patterns are produced by excess biuret and perchlorate (see discussion under Nitrogen, p. 201). Also, the early stages of molybdenum deficiency, as produced in controlled cultures by Vanselow and Datta (1949), were characterized by an apical mottling.

In soil areas where these complications are likely or suspected, leaf analyses should be undertaken to confirm the visual diagnosis. If the symptoms are linked to boron, the total boron of the leaf will probably run higher than 250 to 300 ppm on a dry matter basis, but not infrequently both boron and sulfate may be involved; in some instances, where Chilean nitrate has been used, this also leads to combination symptoms.

In Greece, Demetriades, Holevas, and Gavalas (1962) reported values up to 874 ppm of boron in dry matter of leaves affected by excess boron. Nonchlorotic leaves from injured trees showed up to 505 ppm. Hence, it is possible to find rather a high boron content in leaves which do *not* show symptoms.

On older leaves, particularly orange, grapefruit, and mandarin, there are brown, resinous spots or irregular areas on the undersurface of leaves, where the mottling is caused by excess boron. This latter effect is not seen when sulfate, biuret, or perchlorate is the cause; hence, when the underleaf resinous spotting appears, it helps to confirm the boron excess diagnosis.

Boron accumulates progressively as citrus leaves age; hence, the apical mottling is usually more pronounced in the late summer and fall than in the spring and early summer. As injury progresses, there is necrosis of the leaf tip and margins, and the mottling may affect nearly all of the leaf blade. The boron content of the injured leaf area is higher than in the green portion of the leaves, according to analyses made by Eaton (1935). Eaton found the following concentrations of boron in various parts of injured lemon leaves:

Part of Leaf	*Ppm Boron in Dry Matter*
Midvein and petioles	47
Green portions of blade	438
Yellowed portion	1,060
Dead portion of apices and margin	1,722

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Table 3–15

PRINCIPAL BORON EXCESS EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Boron Excess Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Yellowing of tips, followed by interveinal yellowing (mottling); more prominent at apex (see fig. 3–4).	1. Excess sulfate produces a similar leaf pattern; perchlorate impurity in Chilean sodium nitrate produces a similar, but not identical, apical mottling; early stage of molybdenum deficiency produces an apical mottling similar to boron excess; urea foliage sprays cause a very similar mottling due to the biuret in urea. Leaf analysis will enable differential diagnosis; if boron is less than 150 to 200 ppm, mottling probably is due to something other than boron excess.
2. Sometimes yellow spots appear on upper surface of leaf.	2. Yellow spots on upper sides of leaves sometimes occur with manganese deficiency, chloride excess, and other conditions.
3. Burning of tips and margins of leaves as injury progresses.	3. Injury from excesses of chloride, sodium, potassium, magnesium, ammonium, lithium, and other elements can cause burning of tips and margins of leaves.
4. Brownish, resinous gum spots or pustules on underside of leaf. This symptom usually comes on as the leaf ages. (More common on orange, grapefruit, mandarin, and kumquat than on lemon in Australia.)	4. Sunburn can cause this, but effects are not identical to boron excess.
5. Premature falling of affected leaves. In severe cases, all leaves may be shed; more prevalent in the fall of the year.	5. Many other nutritional disturbances can cause premature falling of leaves.
6. Successive flushes of new growth and shedding of these leaves when injury has become acute.	6. Many other nutritional disturbances can cause successive flushes of new growth.
7. Dieback of twigs, and death of tree in very severe cases.	7. Many other nutritional disturbances can cause dieback of twigs.
Fruiting and Fruit Characteristics	
1. Mild symptoms of injury do not appear to decrease yield. Smith and Reuther (1951) and Scofield and Wilcox (1931) reported that there may be some increase in yield. Severe injury decreases yields.	1. None known.
2. No effect on fruit sizes from slight boron excess (mild leaf symptoms).	2. Information lacking.
3. Little effect on fruit quality from slight boron excess (very mild leaf symptoms); higher boron levels lowered citric acid, vitamin C, total solid, and yields in grapefruit and oranges (Smith, 1955).	3. Information lacking.
Tissue Analysis Values	
1. Total boron in leaves showing various stages of boron excess range from 266.0 to 2,460.0 ppm in dry matter.	1. None known.
2. Necrotic and yellowed portions of leaf show higher boron concentration than uninjured part of same leaf.	2. None known.
3. Twigs and roots do not accumulate boron to the same degree as leaves.	3. None known.
4. In general, the leaf ranges listed below are associated with different degrees of boron deficiency or excess:	4. None known.

Ppm Boron in Dry Matter of Leaf	*Tree or Leaf Condition*
3–15	Acute deficiency
15–25	Acute to slight deficiency
30–100	Normal range
100–300	May or may not be slight to moderate leaf symptoms
200–500	Slight to pronounced leaf symptoms
500–2,400	Pronounced to severe leaf symptoms

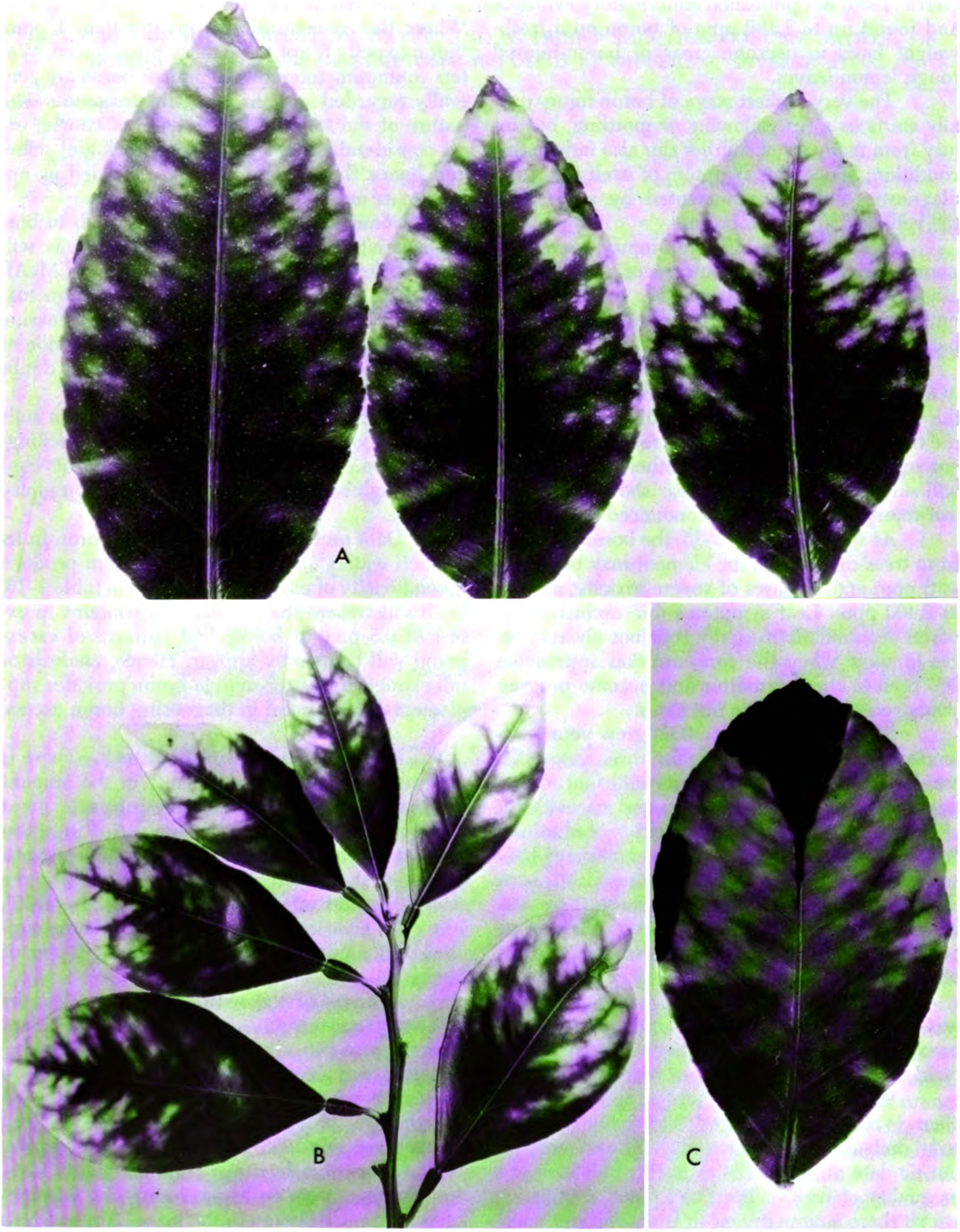

Fig. 3–4. **Boron excess effects:** ***A*****, apical mottling and tip burn on lemon leaves;** ***B*****, apical mottling on orange shoot;** ***C*****, tip and marginal burn and mottling on lemon leaf.**

Oertli (1960) has published confirmatory evidence and found up to 1,180 ppm of boron on a fresh-weight basis in necrotic areas of boron-injured rough lemon leaves.

The very earliest stage of boron injury usually starts as a tip yellowing or mottling, but injury from many other sources can also induce this condition; hence, this symptom of itself is not too diagnostic, unless supplemented by analysis of the yellowed apices for boron.

In severe cases of boron injury, leaves fall prematurely. Successive flushes of growth usually follow; these new leaves are at first green, but subsequently develop typical boron-excess patterns.

Severe leaf injury and abscission eventually result in twig dieback, and, in very severe cases, the entire tree may become defoliated. Death of the tree can occur, but only where the boron present in the soil is very high; otherwise, the tree will survive, but year after year show the typical leaf-injury symptoms and pronounced defoliation.

As shown in table 3–15, the boron of leaves from trees deficient in the element may be as low as 3 ppm and, in cases of severe toxicity, as high as 2,400 ppm. Leaf symptoms due exclusively to boron accumulation usually (but not always) begin to show when the concentration approaches 300 ppm in the dry matter and become progressively acute as the boron increases.

Smith and Reuther (1951) grew young Valencia trees in sand cultures for three years. Excess boron was used in amounts sufficient to produce only mild leaf symptoms, and no evidence of decreased tree growth or yield was obtained. Bradford and Chapman (1964, unpublished) have comparable data. Scofield and Wilcox (1931) state that at the stage of mild leaf symptoms there may be some increase in yield. However, there is insufficient evidence to substantiate this latter observation.

The author is acquainted with many citrus orchards in California where mild symptoms of boron excess appear not to have affected either yield, size or quality of fruit, or tree vigor.

Where boron is present in amounts sufficient to cause pronounced leaf symptoms and twig dieback, tree vigor and yield are reduced, but no data are available as to the effects on size or quality of fruit or the exact point of injury at which there is impairment in the vigor and yield.

Causes of Boron Excess.—Boron excesses in irrigated regions commonly result when irrigation waters contain more than 0.5 ppm of boron. Where the concentration is greater than 1 ppm, boron toxicity is apt to be fairly pronounced. Waters containing more than 2 ppm of boron are generally regarded as unfit for citrus irrigation. The nature of the soil and the climate, of course, exert considerable effect, as do rootstock and scion, and hence these figures can be regarded as approximate only.

Some soil areas are naturally high in boron, and citrus trees growing in these soils will show boron-excess symptoms. Penman and McAlpin (1949) report naturally occurring boron toxicity on citrus in some Australian soils of marine origin. Similar soil areas occur on the west side of the San Joaquin Valley of California, and no doubt elsewhere.

Boron toxicity also has been noted in soils receiving borax wash waters from citrus packinghouses. Some fertilizers and soil amendments contain enough boron to produce severe boron injury of citrus.

Data on the concentrations of boron in irrigation waters and substrate which can produce boron toxicity of citrus are tabulated in table 3–16. With soils where the soil solution contains in excess of 0.5 ppm of boron, leaf patterns of excess boron will commonly appear. Hence, analysis of soil extracts for water-soluble boron provides supplemental data useful in diagnosing boron excess.

Effect of Variety and Rootstock.—There is extreme variability among plants in the amount of boron absorbed from the same substrate, great differences in the amounts accumulating in different parts of the same plant, and pronounced variety and rootstock effects. All of these factors were clearly demonstrated by Eaton and Blair (1935), who grew a variety of plants in sand cultures supplied with a nutrient solution containing 5 ppm of boron.

Analysis of the entire plant revealed the following concentrations of boron in the dry plant tissue: beans, 648; wheat, 453; tomatoes, 159; cotton, 123; and carrots, 69. Eaton and Blair also showed that lemon plants on their own roots accumulated 1,065 ppm of boron in their leaves (dry matter) and only 283 ppm when grown on box orange (*Severinia buxifolia* [Poir.] Tenore) roots. Box orange, grown on lemon rootstocks, accumulated 877 ppm of boron in the leaves (dry matter). On its own roots, it accumulated only 390 ppm in the leaves.

Table 3–16

RELATION OF SOIL, IRRIGATION WATER, AND NUTRIENT SOLUTION CONCENTRATIONS OF BORON TO INTENSITY OF SYMPTOMS AS FOUND BY DIFFERENT INVESTIGATORS

Concentration of Boron in Irrigation Water (Ppm)	Concentration of Boron in Nutrient Medium (Ppm)	Leaf or Tree Symptoms	Concentration of Boron in Leaves (Ppm)	Reported By
	Trace (dry soil)	None	....	Kelley and Brown (1928)
	0.30 (dry soil)	None	....	Kelley and Brown (1928)
	0.50 (dry soil)	Positive	....	Kelley and Brown (1928)
	3.00 (dry soil)	Positive	....	Kelley and Brown (1928)
	21.00 (dry soil)	Positive	....	Kelley and Brown (1928)
50	....	Tree completely defoliated	1,083	Kelley and Brown (1928)
5	....	Severe injury on leaves	....	Kelley and Brown (1928)
1	6.00 (displaced soil solution)	Boron injury on leaves	....	Kelley and Brown (1928)
	0.73 (1.5 saturated extract of soil)[*]	Moderate injury	587	Eaton and Wilcox (1939)
	0.87 (1.5 saturated extract of soil)	Moderate injury	548	Eaton and Wilcox (1939)
	1.04 (1.5 saturated extract of soil)	Moderate injury	469	Eaton and Wilcox (1939)
	1.72 (1.5 saturated extract of soil)	Severe injury	729	Eaton and Wilcox (1939)
	2.00 (culture solution)	Slight effect on leaves	480	Haas (1929*b*)
	2.00 (culture solution)	Effect on lemon leaves	322	Haas (1929*b*)
	0.50 (added twice weekly to sand cultures)	No injury	104	Smith and Reuther (1951)
	2.00 (added twice weekly to sand cultures)	Slight injury	262	Smith and Reuther (1951)
	1.30 to 1.80 (water-soluble boron in soil)	Lemon injury	....	Whetstone *et al.*(1942)
	10.8 (water-soluble boron in soil)	Severe injury	950	Penman and McAlpin (1949)

[*] Water added in amount equivalent to 1.5 times the moisture equivalent.

Eaton and Blair concluded that boron absorption is determined in part by the character of absorbing root cells, the nature of boron compounds accumulating in leaf cells, and the equilibrium between mobile and nonmobile forms of boron within the plant.

In Florida, Roy (1943) found that grapefruit absorbed less boron when grown on sour rootstock than when grown on trifoliate.

Haas (1945*b*) analyzed the leaves of citrus growing on various rootstocks in a rootstock experiment conducted on a uniform soil; he found least accumulation where the stock was sour orange and greatest accumulation on trifoliate orange root. Values of 43 to 48 ppm of boron were found when the stock was sour orange and 119 ppm when it was trifoliate orange. Rough lemon roots produced 75 ppm in the leaf, and boron in the leaves of trees on sweet orange root ranged from 58 to 60 ppm. Smith, Reuther, and Specht (1949) reported results on five-year-old Valencia trees in which the least boron uptake was on sour orange rootstock and the maximum was on Rusk citrange. The boron levels in six-month-old leaves were respectively 67.3 and 94.6 ppm in the dry matter.

Penman and McAlpin (1949), in Australia, found that Washington navel and late Valencia oranges were more affected by boron toxicity when grown on rough lemon (Citronelle) than when grown on sour orange. They were least affected on sweet orange stock. Trifoliate orange stock had about the same rating as sweet orange stock. Cooper and Edwards (1950), working with Valencia orange and grapefruit, found no difference in boron absorption when trees were grown on either sour orange or Cleopatra mandarin rootstock. In later work, Cooper and Peynado (1959*b*) found that *Citrus macrophylla* Hook. rootstock tended to exclude boron, but accumulated chloride; Cleopatra, Tonkan, and Sunki mandarin rootstocks accumulated boron but tended to exclude chloride. *No rootstocks excluded both boron and chloride.* Embleton, Labanauskas, and Bitters (1962) found that young, mature leaves of Frost Nucellar Eureka lemons, growing on various rootstocks, showed the following amounts of boron in the dry matter of leaves: *Citrus macrophylla,* 101; *C. moi,* 164; Rangpur lime, 175; sweet orange, 178; *C. taiwanica,* 191; Yuzu, 196; and grapefruit, 215 ppm. From these data, it is apparent that boron toxicity (as well as deficiency) can be greatly influenced by rootstock.

As regards scion varieties, it generally is observed that the lemon is more susceptible to excess boron than the orange; the order of decreasing susceptibility, as found by Penman and McAlpin (1949) in Australia, is lemon, sour orange, mandarin, kumquat, grapefruit, and sweet orange.

Control of Boron Excess.—Control measures depend to some degree on the cause of boron excess. Where excessive boron is present in irrigation water, the best solution, of course, is to find another source of water. If this is not feasible, then the growth of some more tolerant crop is the only alternative, if the injury is severe enough to prohibit economical citrus growing.

Where injury is only moderate, it can be lessened by using water in sufficient amounts to promote frequent root-zone flushing. Heavier than usual applications of nitrogen fertilizer, especially calcium nitrate (Cooper, Peynado, and Olson, 1958), and moderate liming of acid soils are beneficial.

Where high boron is due to natural soil contamination, irrigation practices which promote root-zone leaching, heavier than usual nitrogen applications, and moderate soil liming if the soil is noncalcareous are helpful.

CALCIUM (Ca)

Calcium Deficiency

Until recently, calcium deficiency of citrus had been produced only under controlled sand- and water-culture conditions. Various descriptions have been published by Reed and Haas (1923*a*, 1923*b*, 1924); Camp *et al.* (1941); Chapman and Kelley (1943); Camp, Chapman, and Parker (1949); Bryan (1940); Brusca and Haas (1956*b*); Chapman (1961, 1966), and Chapman, Joseph and Rayner (1965).

Responses to calcium sprays (2 per cent slaked lime) were reported by DeVilliers *et al.* (1958) on field-grown oranges in South Africa. Mature leaves contained as little as 2 per cent total calcium; the soil contained 0.5 meq calcium per 100 gm; and trees showed sparse foliation, twig dieback, chlorosis symptoms, profuse blossoming, and excessive drop of young fruit. Fruit which matured was often misshapen, acid, and on the flat side as regards total solids. DeVilliers *et al.* (1958) noted that there were several areas in South Africa where calcium was low, and thus there was the possibility of calcium deficiency showing up. In Florida, Spencer (1960) and Spencer and Koo (1962) uncovered calcium deficiency on Ruby Red grapefruit (rough lemon root) and on Pineapple orange growing in a Lakeland fine sand. The trees which developed calcium deficiency were on acid check plots of a fertilizer experiment involving limestone and superphosphate. No calcium deficiency developed on either the limestone or superphosphate plots. The calcium-deficient trees were stunted and produced much less fruit than those receiving calcium. A leaf chlorosis developed, characterized by irregular marginal yellowing, with the midribs and veins and areas surrounding the veins retaining their green color. These symptoms were much the same as those illustrated by Chapman and Kelley (1943, fig. 160, p. 731) and by Bryan (1940) (see fig. 3–5). The calcium content of six-month-old leaves from nonfruiting terminals, as reported by Spencer and Koo (1962), ranged from 0.80 to 1.23 per cent in the dry matter, as compared to 3.17 per cent for comparable leaves from non-calcium-deficient trees.

As reported by Spencer and Koo (1962), analyses of late-summer-flush grapefruit leaves sampled in January from trees lacking calcium compared with similar leaves from healthy trees were as shown below.

Treatment and Condition	*Percentage in Dry Matter of Leaves*					*Ppm in Dry Matter of Leaves*			
	Ca	N	P	K	Mg	Fe	Mn	Zn	Al
+Ca — green	2.47	2.26	0.14	2.06	0.28	60	27	19	46
−Ca — chlorotic	0.24	1.97	0.12	2.96	0.30	47	15	21	53

Table 3–17

PRINCIPAL CALCIUM DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Calcium Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Tree growth is stunted and tops tend to round off. Loss of vigor, short growth flushes, dieback, and thinning of foliage become progressive.	1. Tree stunting and rounded tops can be produced by copper deficiency, salt injury, virus diseases, and other factors.
2. Midribs on leaves appear lighter in color; may be more pronounced in winter, improving with warmer weather.	2. Boron deficiency can produce lighter vein colors; also girdling of trunk or branches.
3. Vein chlorosis and leaf yellowing occur in more acute stages as a result of root rotting. Lack of calcium may make roots more susceptible to decay.	3. Root rotting, bark diseases, nitrogen deficiency, senescence, cold weather, and virus diseases can all produce more or less vein chlorosis, though in varying degrees and circumstances.
4. Midribs of some leaves are shortened, and apex of leaves may be blunted and rounded; leaves may become heart-shaped.	4. Salt injury and other deficiencies may cause leaves to become more rounded.
5. As deficiency becomes worse, leaf margins become yellowish (with veinal areas remaining greener) and patterns develop (see fig. 3–5).	5. Boron, sodium, and manganese excesses and magnesium deficiency can produce somewhat similar patterns.
6. Where potassium is high, necrotic spots appear only on upper surfaces of leaves (see fig. 3–5). Calcium in dry matter may be less than 0.5 per cent, and potassium, over 2.5 per cent.	6. Author has not seen this particular condition under any other circumstance.
7. Leaf sizes are reduced under calcium deficiency (Brusca and Haas, 1956*b*).	7. Many other factors reduce leaf size.
Fruiting and Fruit Characteristics	
1. Yields are decreased.	1. Reduced yields occur under many conditions.
2. Fruit sizes are reduced, especially if magnesium is high; where potassium is high, fruit sizes may be increased.	2. Reduced fruit sizes occur under many conditions.
3. If magnesium is high and potassium is low, rinds are smooth; fruit may be misshapen and juice vesicles shriveled or gelatinized (see fig. 3–6).	3. Boron and sulfur deficiencies produce somewhat similar effects.
4. There are no highly specific fruit conditions which are sufficiently diagnostic by themselves to clearly permit calcium deficiency diagnosis.	4. Nitrogen and several other deficiencies do not have highly diagnostic fruit symptoms.
Tissue Analysis Values	
1. Mature, 4- to 7-month-old leaves with less than 3.0 per cent calcium in dry matter would be suggestive of calcium deficiency. Acute stages show calcium values of less than 1 per cent, and magnesium and potassium values are usually higher than normal.	1. No other factors known.

Accompanying the marked decrease in calcium was a decrease in nitrogen, iron, and manganese. Potassium and magnesium, on the other hand, were increased.

In the Florida trees, premature leaf drop, multiple buds, and dieback of the trees occurred. Some of the leaves were heart-shaped due to shortened midribs. Some of the fruit was misshapen from shrunken juice vesicles. The juice was more acid.

These symptoms correspond well with those produced under controlled conditions by Chapman and Kelley (1943) and Bryan (1940), and with those described by DeVilliers *et al.* (1958) on oranges in South Africa. A summary of the various growth, foliar, fruit, and leaf analyses conditions which characterize calcium deficiency on citrus, as produced both under controlled conditions and in the field, is presented in table 3–17. Illustrations of tree, foliar, and fruit symptoms are shown in figs. 3–5 and 3–6.

Causes of Calcium Deficiency.—The two field cases of calcium deficiency reported thus far —in South Africa and Florida—were on soils of

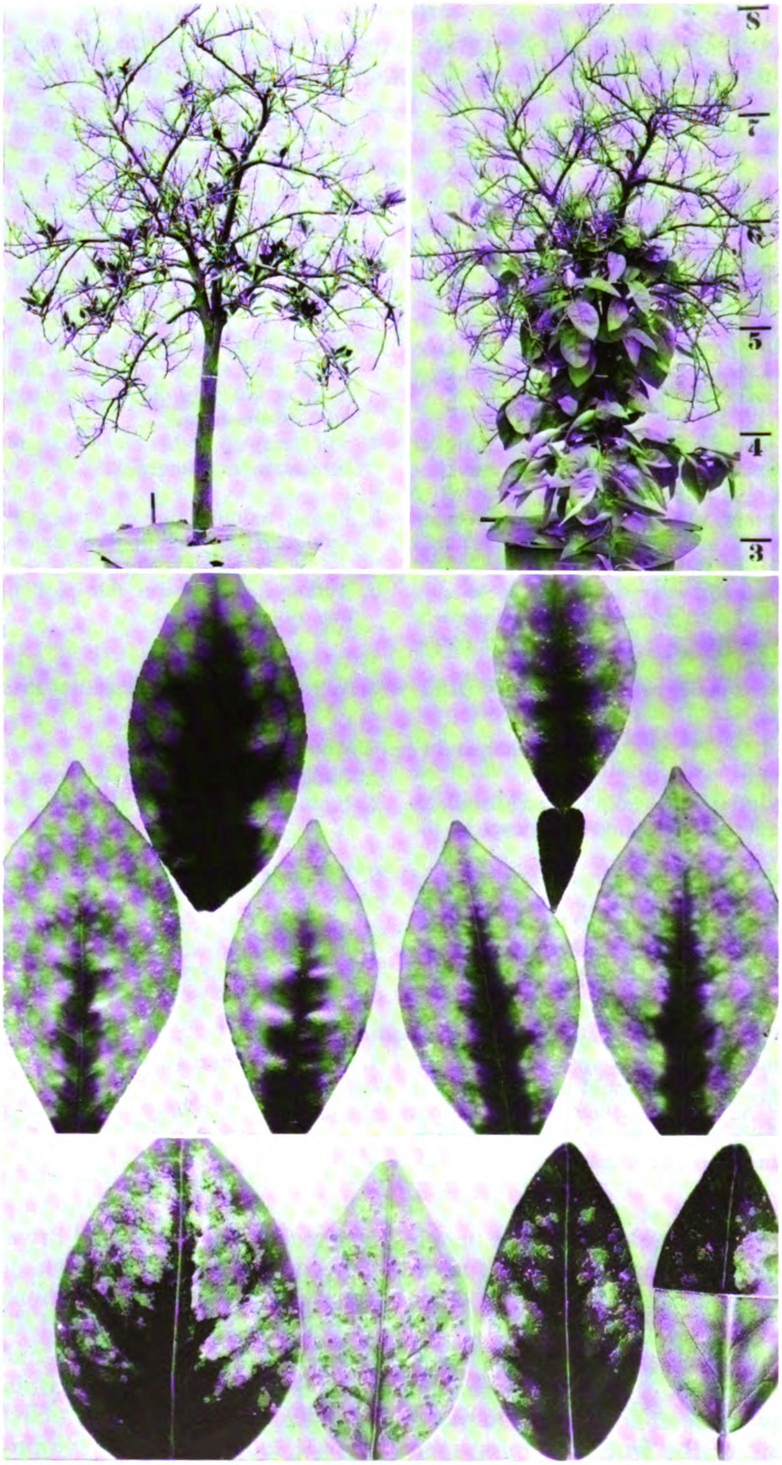

Fig. 3–5. Calcium deficiency effects on orange tree growing in sand culture. Top left, severe defoliation and dieback. Top right, same tree showing new, vigorous growth after calcium was restored to nutrient solution. Middle, marginal-type chlorosis produced in moderate to severe stages of calcium deficiency. Bottom, necrotic spots characterize upper sides of leaves when calcium is very low and potassium is high.

very low calcium content. The South African soil had 0.5 meq of calcium per 100 gm (or 100 ppm), while the Florida soil showed 8 to 30 ppm on soils of pH 4.3 to 4.5. Recently, Martin and Page (1965) produced calcium deficiency of sweet orange seedlings in potted soils in which the exchangeable calcium percentage was reduced progressively. When the percentage calcium saturation of the soil was at about 15, low leaf levels of 0.5 to 0.7 per cent were produced in the dry matter. No consistent leaf patterns developed, however.

The author and his colleagues (Chapman, Joseph, and Rayner, 1965) recently concluded an eight-year experiment on the calcium nutrition of bearing orange trees (Troyer citrange rootstock) growing outdoors. Moderately acute calcium deficiency occurred in cultures where the calcium concentration ranged from 10 to 20 ppm in the nutrient medium. Only slight deficiency developed on trees in cultures containing 40 to 50 ppm of calcium. While the calcium deficiency was not acute enough to produce marginal yellowing of leaves, some vein clearing was noted on the foliage, trees were stunted, and growth flushes were shortened. Manganese deficiency occurred in the winter, and some root rotting was noted in container areas remote from the aerator tube. In similar areas of the culture container where calcium was higher, no root rotting occurred. This strongly suggests that roots under subnormal calcium conditions are more susceptible to fungi invasion than those whose calcium nutrition is good. (*Fusarium* spp. were the chief fungi.) This may explain the rather widespread, vein-chlorosis condition which the author has seen in parts of Australia, India, and elsewhere. Poor aeration (high moisture), low calcium, low nitrogen, cold soil temperatures, and viruses may all be involved in vein chlorosis also.

The results suggest that while acute calcium deficiency of citrus is not likely to be encountered except in sandy, acid soils of low-calcium content, low-calcium conditions may be more widespread than previously supposed. Trials with calcium salts or limestone should be initiated wherever the calcium content of mature, four- to six-month-old leaves is less than 3 per cent.

Low-calcium conditions, while most common in acid soils, may possibly occur under conditions of high soluble sodium or pH conditions resulting from exchangeable sodium percentages well above usual levels. Calcium is important in counteracting the effects of high soluble sodium levels and may be of such low solubility in soils dominated by exchangeable sodium as to bring on calcium deficiency.

Low-calcium conditions have been reported on serpentine-derived soils and strongly acid peats. Calcium absorbed on kaolinite appears more available to plants than that absorbed on montmorillonite.

Control of Calcium Deficiency.—The Florida field case of calcium deficiency, as already mentioned, was corrected by soil treatment with limestone and/or superphosphate. In South Africa, correction was obtained by a foliar spray of 2 per cent lime hydrate. The worldwide successful use of limestone and/or gypsum for correcting

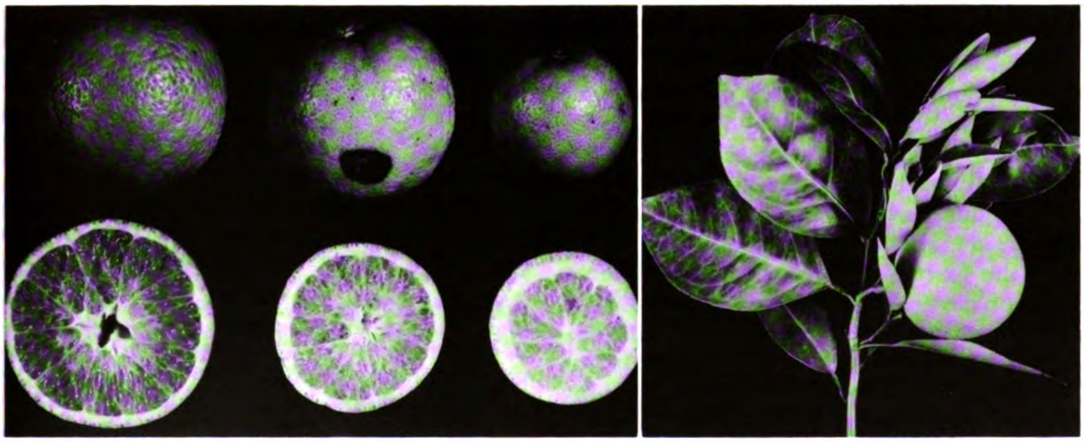

Fig. 3–6. Effects of calcium deficiency under low-potassium conditions on orange fruits and foliage. Left, fruits show shriveling and gelatinization of juice vesicles, smooth rinds, rind spotting, and a tendency to be misshapen; fruits are smaller than when calcium is present. Right, in moderate deficiency, leaves (especially in winter) show some vein clearing; fruit production and sizes are decreased.

low calcium and calcium deficiency under a broad range of conditions indicates that no serious correction problems are encountered with citrus when deficiency or low-calcium conditions occur. Sand-culture studies have shown that trees almost dead from lack of calcium recovered rapidly when this element was restored.

Calcium Excess

Calcium excess effects are due more to the anion with which it is associated than to the calcium ion per se. In the case of soluble salts, such as calcium chloride or sulfate, it appears to be the excessive absorption of chloride and/or sulfate that produces trouble rather than the accumulation of calcium in tissues. However, more research needs to be carried out on possible specific effects of high-calcium concentration.

In the case of calcium carbonate, the alkalinity and buffering effects it produces in soils and the effects of these on the availability of manganese, iron, zinc, copper, boron, and phosphorus are of principal importance.

COPPER (Cu)

Copper Deficiency

In Florida, a citrus condition first described as "dieback" or "red rust" was reported by Fowler (1875). Floyd (1914) found that copper Bordeaux sprays corrected the condition, and Grossenbacher (1916) found that soil applications of copper sulfate restored affected trees. However, it was not until 1925 and after that copper was fully accepted as an essential element for plants. This acceptance was preceded by the research of McHargue (1925) on copper distribution in plants and animals, Bortels' work (1927) with *Aspergillus niger,* the work of Felix (1927) and Allison, Bryan, and Hunter (1927) on responses of various crops to copper additions to peat soils, and the more exact work of Sommer (1931) and Lipman and MacKinney (1931). Haas and Quayle (1935) were probably the first to produce copper deficiency symptoms on citrus. Valencia orange trees on sour rootstock were grown in large outdoor sand cultures from 1920 to 1928. By 1926 the trees which had been supplied with nutrient solutions lacking copper developed the symptoms then described as exanthema. Additions of copper sulfate corrected the condition on all but one of the trees. During this same period, somewhat comparable observations and research led to the conclusion that zinc, boron, and manganese were also essential for plant growth. Thus, much of the previous evidence fell into place. Wade (1944) later produced copper deficiency of citrus in controlled water cultures, and Arnon and Stout (1939*a*) produced copper deficiency in tomato. Copper deficiency in plants has been widely reported. Good reviews of copper in relation to plant and animal nutrition are given by Gilbert (1952), McElroy and Glass (1950), Reuther (1957), Reuther and Labanauskas (1966), and others.

Symptoms of Copper Deficiency.—The growth, foliar, and fruit symptoms characterizing copper deficiency of citrus have been well described by Camp, Chapman, and Parker (1948, p. 314) as follows:

> The first evidence of mild copper deficiency is the occurrence of unusually large, dark-green leaves on long, soft, angular shoots, the leaves being commonly of irregular contour, usually with a "bowing up" of the midrib. The soft twigs sag at the tips or become S-shaped. In this stage the tree appears to the casual observer as unusually vigorous, although in California this excessive growth is not so prominent. When the deficiency is more acute, very small leaves may develop and quickly shed on twigs that are going to die back, but on the older wood the leaves will be large, dark green, and somewhat twisted or malformed. This peculiar twisting and malformation of foliage is particularly common on copper-deficient grapefruit trees. In very acute cases the leaves may be greatly distorted, the margins irregular, and the color light green with a fine network of darker green veins. In such cases the growth of the twigs is restricted, fine, and very angular.
>
> Following the appearance of the initial symptoms, the affected twigs usually show a development of multiple buds. These produce a dense, somewhat bushy growth, particularly in lemon trees of moderate vigor. Occasionally gum pockets develop between the bark and the wood. These may rupture the bark and permit the gum to exude. In dry weather the gum collects and dries on the surface of the bark. However, it is readily soluble in rain water and frequently is overlooked for that reason. As the deficiency becomes more acute, new growth develops and then dies back for several inches. In very acute cases, heavy twigs having multiple buds put out a profusion of young soft shoots with small leaves, which quickly die back from the tips. In this stage the twigs have a reddish excrescence over a large portion of the bark. Neither the dying back nor the reddish excrescence is so pronounced in grapefruit as it is in oranges, and it is still less obvious in tangerines. As the acute stage is reached, there is a pronounced loss of growth due to dying back, and in very serious cases the tree may be almost killed. The dying starts on the outer shoots of the tree; soon the characteristic large soft shoots appear as water sprouts on large branches in the center of the tree, and these in turn develop the characteristic symptoms. In acute stages gumming has been found in the roots also, and considerable loss of roots takes place.

In cases of severe copper deficiency, the fruit is marked irregularly with reddish-brown excrescences, which are light-colored on young fruit but progressively darken until they may be black on mature fruit. Young fruits are sometimes bumpy and generally have an unusually light green color and a very smooth skin with or without the light reddish-brown excrescences. By June some of the fruits will be almost covered with these excrescences and drop from the tree. Such fruits as are left usually have juice low in acid and very insipid, and the pulp dries out early in the season. In such acute cases there are gum pockets in the rind and gum at the axes of the segments; splitting of young fruits is common and includes both the ordinary longitudinal splitting with starts at the stylar end and transverse splitting which starts in an excrescence and extends part way around the fruit.

The symptoms described are typical of oranges. In grapefruit, the excrescences on the peel are less common, and though gum pockets are numerous in the rind, gum is seldom found around the seeds. Fruits from copper-deficient grapefruit trees are commonly yellower than normal and are frequently lopsided, and as they mature brown pits develop in the rind that are similar to storage pits but smaller. Acute copper deficiency is rather uncommon in grapefruit and seldom observed in tangerines.

Acute deficiency of copper may put trees entirely out of production, while in the intermediate stage there is a reduction in yield and also considerable loss due to lowered grade of the fruit.

Copper deficiency tends to reduce the soluble-solids content and also the acid content of orange juice. Since the reduction in acid tends to be disproportionate to the reduction in solids, the fruit from deficient trees tends to have a higher ratio of solids to acid. The vitamin C content of the juice is also reduced, more or less in proportion to the reduction in acid. It is interesting to note that this reduction in solids and acid and also vitamin C varies with different varieties as it relates to the occurrence of other symptoms. The Hamlin variety of orange may show a very high reduction in solids and acid with very few of the visible morphological symptoms, whereas in other varieties the extent of the symptoms more or less parallels the reduction in the soluble constituents of the juice.

Zinc deficiency is commonly associated with copper deficiency in Florida. This results in some modification of the growth symptoms owing to the growth-restraining characteristics of zinc deficiency. There is also considerable evidence to indicate that copper deficiency restrains absorption of zinc by the roots, so that in acute cases of copper deficiency, symptoms of zinc deficiency almost always develop even though the amount of available zinc in the soil is adequate for healthy trees.

Table 3–18 summarizes some of the foliar, growth, and fruit symptoms of copper deficiency and includes tissue analysis values.

Illustrations of various copper deficiency effects on fruits, shoots, and foliage are shown in figures 3–7, 3–8, and 3–9.

Causes of Copper Deficiency.—In Florida, copper deficiency occurs prominently on leached, acid sandy soils, on peat soils, or even on those soils high in organic matter. It is common in young plantings on virgin soils.

In California, the condition (while much less prevalent than in Florida) has been noted on alkaline sandy soils and on heavy soils rather high in organic matter; also, along with severe zinc deficiency, it has been noted in old corral sites and Indian burial grounds. This may be due to the accumulation of phosphorus, since Bingham *et al.* (1958) showed that heavy phosphate fertilization can induce copper deficiency. In field phosphate experiments, Reuther *et al.* (1949) found that foliage levels of copper were reduced by heavy phosphate treatments.

In Australia, copper deficiency has been noted on sands, sandy lateritic and sandy calcareous soils, and acid muck soils (Teakle and Stewart, 1939; Teakle, 1942).

Excessive nitrogen fertilization has been considered as a cause of copper deficiency, especially in Florida. Insufficient copper in the soil or a supply which is quickly exhausted by cropping is considered a prime cause of the formerly widespread copper deficiency of citrus on the acid sands of Florida. Bryan (1958) reported that, in Florida sandy soils, from 3 to 5 ppm of total copper are sufficient for citrus where phosphate is high and that 1 ppm is ample in low phosphate soils. He further stated that 10 ppm of copper are excessive. Dr. P. F. Smith of Florida, in private correspondence with the author, stated that virgin Florida soils vary from 2 to 15 ppm total copper, and that toxicity begins around 100 ppm and over. Copper forms highly insoluble compounds with organic matter and is tenaciously fixed by clays in forms not readily replaceable.

Liebeg *et al.* (1942) found, in controlled culture work, that aluminum reduced copper toxicity, and, by the same token, soil conditions could obtain (e.g., acid soils) where copper deficiency might be produced by soluble aluminum. No doubt many other specific interrelations will come to light as knowledge in this field advances.

Copper Excess

The toxicity of copper to microorganisms has been known for many years, and copper has been used in various formulations as a fungicide. Bordeaux spray originated in 1882 near Bordeaux, France. The toxic effects of copper on higher plants became known before the turn of the century. (For references on much of this early work, the reader is referred to the *Bibliography of the*

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Table 3–18

PRINCIPAL COPPER DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Copper Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. In some cases, first evidence is large, dark green leaves on long, soft, angular shoots; leaves are commonly irregular in contour, with bowing of midrib. On these succulent shoots it is not uncommon to find gum boils or gum pockets in the woody tissue and between the bark and wood (see fig. 3–7).	1. Sucker growth often occurs on young, vigorously growing citrus, but gum boils and pockets are not present.
2. Dieback of terminal shoots with reddish to brownish to blackish gum deposits on bark; multiple buds form and a profusion of shoots appear with small leaves; these shoots die back. The term "red rust" is sometimes used to describe gumming on these shoots.	2. Dieback of terminal shoots occurs where calcium, boron, phosphorus, zinc, and other deficiencies exist, but it is not common to see the gum staining of the bark in these cases.
3. S-shaped shoots may be seen.	3. It is not uncommon to find S-shaped twig growth occuring more or less promiscuously on most any citrus tree, especially where rapid growth is occurring.
4. Copper-deficient trees sometimes show a rather flattish top due to arrested growth; trees may appear somewhat dwarfed.	4. Salt injury may cause arrested or decreased growth, as well as many other adverse soil or climatic factors.
5. On young, succulent shoots, it is possible on occasion to see beads of gum which ooze out from cracks in the bark. These beads of gum wash off easily (see fig. 3–8).	5. Cracks in bark occur from frost damage; red scale infestation may cause exudation of gum on branches and twigs.
6. A finely netted venation or flecking on young developing leaves may sometimes be seen (see fig. 3–8).	6. Somewhat similar to iron chlorosis.
7. Zinc and copper deficiencies often occur together.	
Fruiting and Fruit Characteristics	
1. Brownish to reddish or blackish excrescences of gum on rind of fruit; this condition may or may not be associated with a scabby appearance and small cracks in the rind irregularly spread over the rind surface (see fig. 3–7).	1. Tear staining, resulting from *Septoria,* rust mite damage, and wind and hail, causes a variety of symptoms resembling in some cases those associated with copper deficiency.
2. Gum pockets are commonly seen around pith in cut sections (see figs. 3–7 and 3–8).	2. No other condition known to author produces such symptoms.
3. Small, dwarfed fruits with more-or-less silver or light green rinds and/or gum excrescences may be seen. Often such fruits show gum pockets around pith and have dried-up interiors (see fig. 3–8).	3. Dwarfing and silvering are seen in sulfur deficiency, but not the rind excrescences of gum or the gum on the interior of the fruits.
4. In grapefruit, gum pockets may be seen in the albedo and also in the fleshy part of the fruit, not necessarily around the pith. Fruits are often misshapen, with coarse rinds and brown pits on rind surfaces (see fig. 3–9).	4. Boron deficiency commonly produces similar symptoms in both orange and grapefruit. Differential diagnosis may be made by leaf analyses for copper and boron.
5. Yields are markedly decreased.	5. Yield decreases are common from other deficiencies, such as zinc.
6. Acid and vitamin are reduced.	6. Excess phosphate produces the same effects.
Tissue Analysis Values	
1. Copper content in leaves showing typical copper deficiency effects commonly is in the range of 2 ppm or less in the dry matter. However, values as low as 2 ppm are sometimes found in good-producing, healthy orchards (Bradford and Harding, 1957). In general, the majority of healthy orchards show values in the range of 4 to 10 ppm of copper.	1. None known.

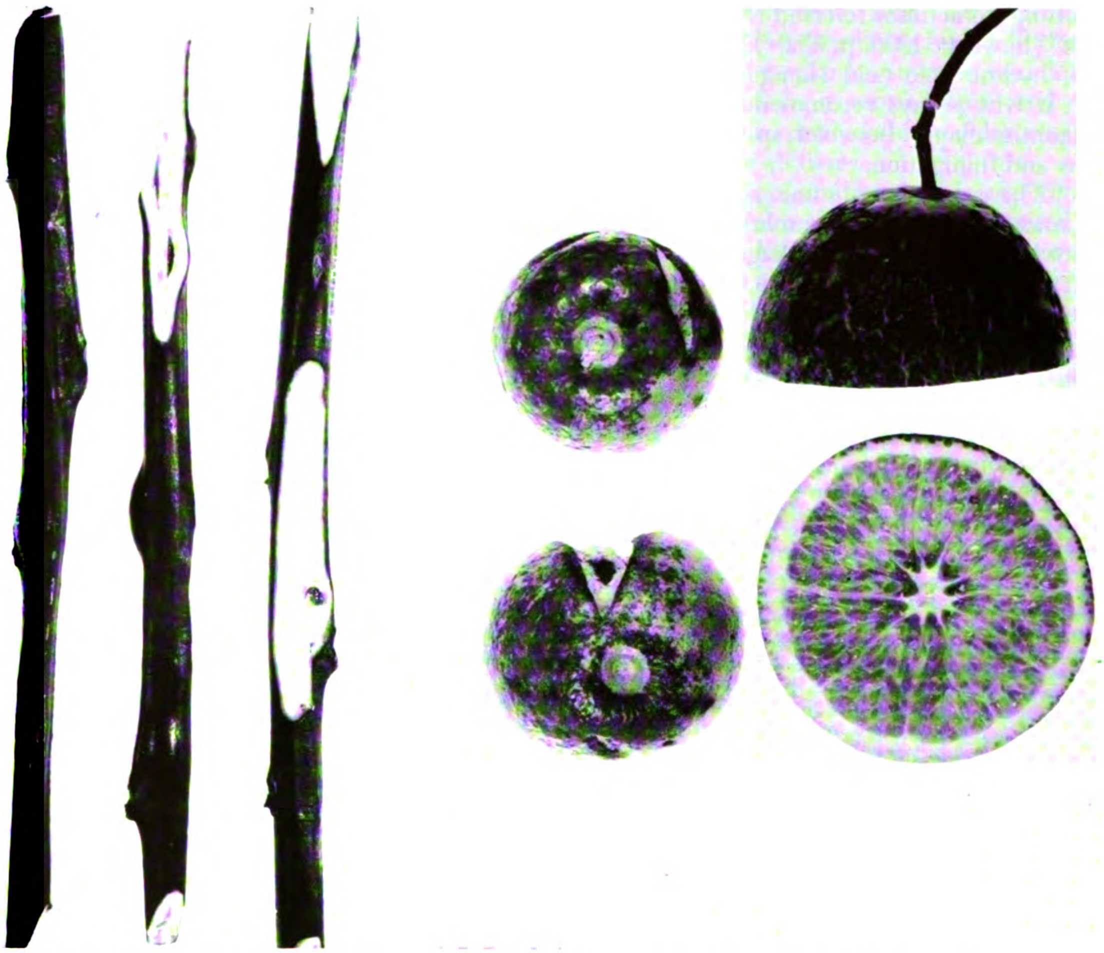

Fig. 3–7. Effects of copper deficiency on orange. Left, sucker growth showing gum blisters and gum pockets. Top right, mature orange fruit showing gum excrescences on rind and splitting tendency. Lower right, mature orange fruit showing scabby, reddish-brown, cracked appearance of rind, gum pockets around central pith, and semigelatinized juice vesicles.

Literature on the Minor Elements, published by the Chilean Nitrate Educational Bureau, Inc., New York, and to Forbes, 1917).

In general, copper excess problems have arisen chiefly from the excessive use of Bordeaux sprays or through soils, streams, and irrigation waters becoming enriched or contaminated by copper mining operations.

In Chile, the author saw some areas where copper injury had developed from mining operations; also, some streams were pointed out that contained elevated copper concentrations as a result of water percolation through copper-bearing rocks.

As far as citrus is concerned, toxic effects to the tops from repeated applications of Bordeaux sprays have been commented upon by Fawcett (1936, pp. 358–59). He noted that injury was more likely to occur from repeated sprayings in summer, especially in interior valleys, and that affected trees gradually lost many leaves, though no spotting or burning took place. Yields were decreased and many small twigs died. However, no permanent injury resulted. Scale-insect infestations increased, probably as a result of the killing of fungus parasites.

The use of Bordeaux sprays, followed too closely by hydrocyanic acid fumigation, has often been noted to result in injury. In these cases, severe defoliation may take place. Various kinds of leaf burn may occur, such as burned spots or death of the entire blade; there also may be only a spotting, sometimes referred to as "stellate melanose" (Fawcett, 1936, p. 552). During the period when hydrocyanic acid fumigation was widely used, it was always recommended that at least six months elapse between spraying with Bordeaux and fumigation.

There has been some reason to believe that smog components can solubilize the copper in Bordeaux residues on foliage and bark and cause injury and that moisture condensation on leaves, perhaps coupled with carbon dioxide formation from honeydew, may solubilize copper sufficiently to cause injury. Discussion of these and other possibilities has been presented by Klotz, Calavan, and DeWolfe (1956). These investigators recommend the incorporation of zinc sulfate to offset copper spray injury in areas subject to such trouble. A formula consisting of 3 pounds of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$), 2 pounds of copper sulfate ($CuSO_4 \cdot 5H_2O$), and 6 pounds of hydrated lime ($Ca(OH)_2$) is satisfactory.

Another type of injury resulting from copper accumulations in the sandy acid soils (pH 4 to 5.5) of Florida—after repeated use of Bordeaux sprays and soil applications of copper sulfate—has been described by Reuther and Smith (1953, 1954) and Reuther (1957). Chlorotic foliage of low-iron content, marked reduction in vigor and yield, and dieback of twigs were some of the effects on the above-ground parts of the tree. Fibrous roots growing in the zone of high-copper content were sparse, dark, and stubby. Such soils commonly contain from 100 to 400 ppm of total copper in surface layers, as compared with values of less than 5 ppm in soils where Bordeaux sprays or soil applications have never been used (Reuther *et al.*, 1951; Reuther, Smith, and Scudder, 1953; Wander, 1954).

Iron chelates (e.g., FeEDTA) at rates of ¼ to ½ pound per tree, coupled with lime to increase alkalinity to about pH 7, will correct the condition. The lime reduces copper solubility, and

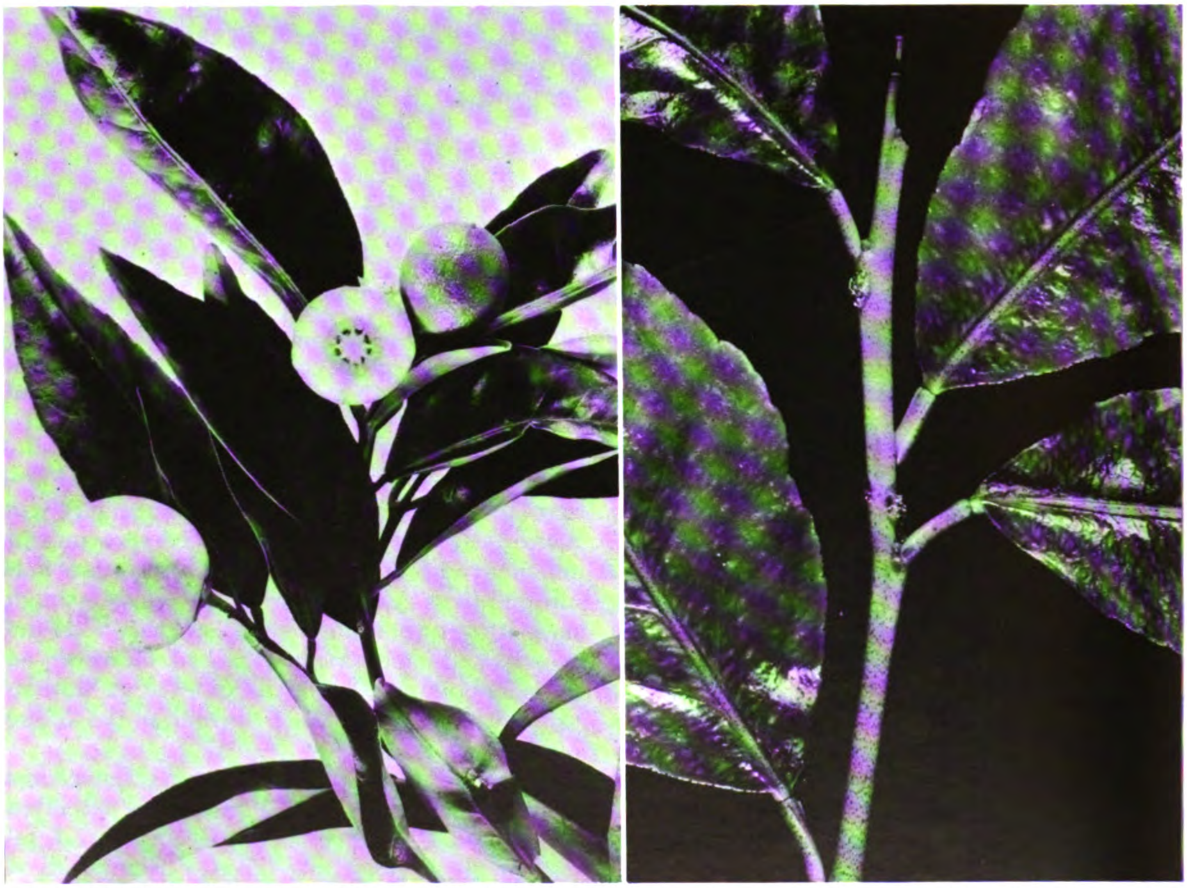

Fig. 3–8. Effects of copper deficiency. Left, orange twig showing small, silvered fruits with interior gum pockets. Right, young lemon shoot showing beads of gum and a stippled mottling on the leaves.

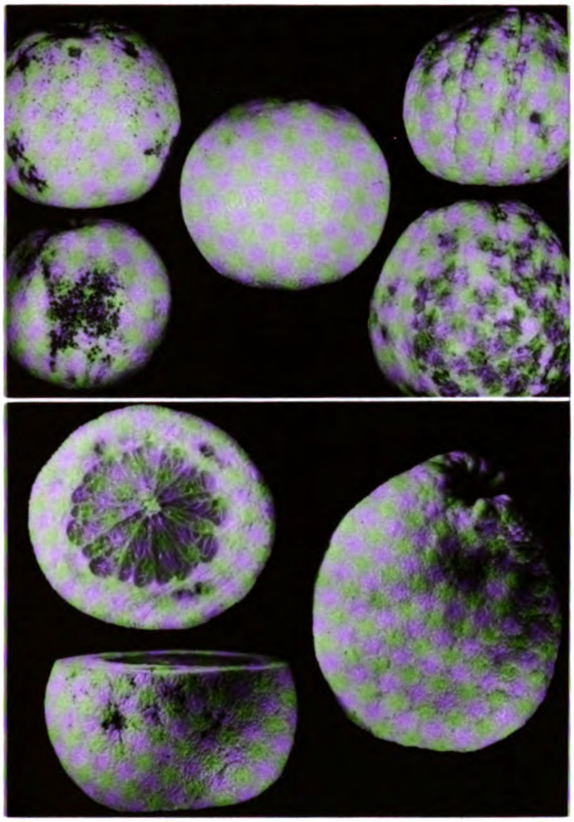

Fig. 3–9. **Effects of copper deficiency on grapefruit. Top, healthy fruit in center. Affected fruits are rough, misshapen, and have brown spots and excrescences on the rinds. Bottom, affected fruits showing gum spots in the albedo, thick rinds, and off-shape. (Photos courtesy of Bradford, Harding, and Miller, 1964.)**

the chelate furnishes iron to the nonaffected roots in lower soil horizons. Phosphate or lime will also decrease copper toxicity.

In culture work, Forbes (1917) noted that citrus seedlings placed in distilled water to which 2.5 ppm of copper were added wilted within 48 hours. Chapman, Liebig, and Vanselow (1940) and Liebig *et al.* (1942) noted that excessive copper in nutrient solutions sometimes produced iron chlorosis. The latter investigators found that as little as 0.1 ppm of copper in the nutrient solutions markedly affected the roots and tops. Swollen, short-root laterals and brown color characterized the roots of the copper-injured plants. The injured fine roots showed up to 890 ppm of copper, as compared to less than 100 ppm in healthy roots. It was found that aluminum at concentrations comparable to the copper would offset the toxic effect of the copper (see fig. 3–10).

Smith and Specht (1952, 1953) produced iron chlorosis in citrus seedlings from additions of copper (0.1 ppm) to plants grown in vermiculite. Higher concentrations of zinc (3 ppm) and manganese (5 ppm) also produced iron chlorosis. Root injury occurred in all cases.

In short-term, solution-culture experiments with wheat, Blevins and Massey (1959) found that 0.4 to 1 ppm of aluminum decreased copper uptake, but that 0.1 ppm of aluminum appeared to increase copper absorption. Copper concentration in the nutrient solution was 0.1 ppm. In pot-culture experiments with soil, an inverse correlation was noted between soil aluminum, extracted by 0.1*N* sodium chloride, and copper uptake by millet.

In work with corn, Willis and Piland (1936) found that copper sulfate added to a peat soil produced iron chlorosis.

Neither plant appearance nor leaf analysis can be relied upon as infallible guides to copper excess effects. Table 3–19 summarizes some of the principal effects of excess copper on citrus trees and roots. Unlike boron and some other elements, copper does not always accumulate in sufficient amounts in leaves to afford a reliable indication of copper toxicity; but in cases of suspected injury, leaf analyses should be made.

Where soil contamination is suspected, analyses of both rigorously cleaned fine roots and soils should be made. Total copper concentrations of 50 to 100 ppm or over in the soil should be regarded with suspicion. Copper levels of over 15 to 20 ppm in leaves should also be regarded as indicating possible copper toxicity. In pot-culture work with citrus seedlings, Reitz and Shimp (1954) noted that decreased growth from additions of either copper oxide or copper sulfate was associated with over 20 ppm of copper in the dry matter of the leaves.

Roots injured from excess copper may show from 400 to more than 900 ppm of copper in the dry matter (Liebig *et al.*, 1942; Smith and Specht, 1952, 1953).

In recent experiments by the author (unpublished) with young navel orange trees on Troyer rootstock, copper in leaves up to 18 to 20 ppm in the dry matter was associated with marked yield reductions though tree appearance and growth were not appreciably affected. High calcium concentration (from gypsum) reduced copper intake, and at leaf levels of 10 to 12 ppm of copper, good crops of fruit were matured.

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Table 3–19

PRINCIPAL COPPER EXCESS EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Copper Excess Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Splitting of bark and gumming from either excessive soil applications or from Bordeaux spray residue accumulations in acid soils.	1. Gumming and bark splitting can occur where red scale infestation is severe. Copper deficiency often produces some bark splitting and gumming; boron and potassium deficiencies can also produce these effects.
2. Marked defoliation and even death of the tree can occur where injury is severe.	2. Sudden defoliation can, on occasion, occur from salt injury; severe red spider damage, coupled with moisture stress; or other severe shocks to the tree.
3. In nutrient cultures, as little as 0.10 ppm copper in solution can, under some conditions, cause damage to roots (see fig. 3–10).	3. Similar root growth effects can be caused by many other factors. Root and soil analyses make differential diagnosis possible.
4. Excess copper in soils will often induce iron chlorosis, especially in Florida, where copper has accumulated from Bordeaux sprays and bluestone added to soils.	4. Iron chlorosis can be produced by low iron availability (as in calcareous soils), excess zinc, and other heavy metals, over-moist soil conditions, low soil temperatures, and other causes. (See section on iron, p. 161).
5. Severe leaf burn and defoliation can occur when HCN fumigation follows too closely after spraying with copper Bordeaux mixtures. Leaves may abruptly drop from the tree or show large or small burned spots.	5. HCN fumigation will cause some leaf drop but not as severe as that produced when it follows too closely after copper Bordeaux spraying.
6. Repeated spraying with Bordeaux can cause reduced growth, some defoliation, decreased yields, decreased fruit sizes, and increased scale infestation.	6. Salt injury and many other conditions can produce defoliation, reduced growth, and reduced yields.
Tissue Analysis Values	
1. Copper in amounts exceeding 15 to 20 ppm in the dry matter should be looked on with suspicion and followed by soil analysis. Yields may be markedly decreased with only slight excesses of copper.	1. It is probable that acute phosphorus and other deficiencies will lead to increased copper levels in leaves.

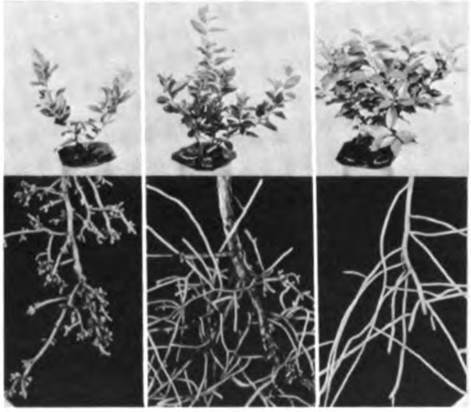

Fig. 3–10. Copper excess effects. Left, reduced top growth associated with severe root injury from presence of 0.1 ppm copper in the nutrient medium. Center, root and top growth in culture containing approximately 0.05 ppm copper in the nutrient medium. Right, amelioration of copper toxicity effects by addition of aluminum to nutrient medium.

FLUORINE (F)

There is no evidence that fluorine is essential for citrus or any other green plant, but its wide distribution in soils, plants, and waters, and its effects on plants and animals have been the subject of special study over many years.[1]

Flourine Excess

Possible fluorine toxicity to citrus first came to the author's attention in 1951, when leaf-scorch of apricot trees, comparable to that described by DeOng (1946) from fluoride deposits on leaves, was found near a steel plant in San Bernardino County, California (McCornack *et al.*, 1952). Citrus growers in the area became alarmed over excessive leaf drop and poor yields. Preliminary analyses of citrus leaves from trees near the steel plant showed much higher fluorine than did leaves from trees remote from industrial contaminants. This led to an extensive leaf analysis survey of

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[1] A key to the early literature on fluorine may be found in Volume 1 of the *Bibliography of the Literature on the Minor Elements*, published in 1948 by the Chilean Nitrate Educational Bureau, Inc., New York. A more recent review, with special reference to effects of fluorine air pollutants on plants, has been given by Thomas (1961).

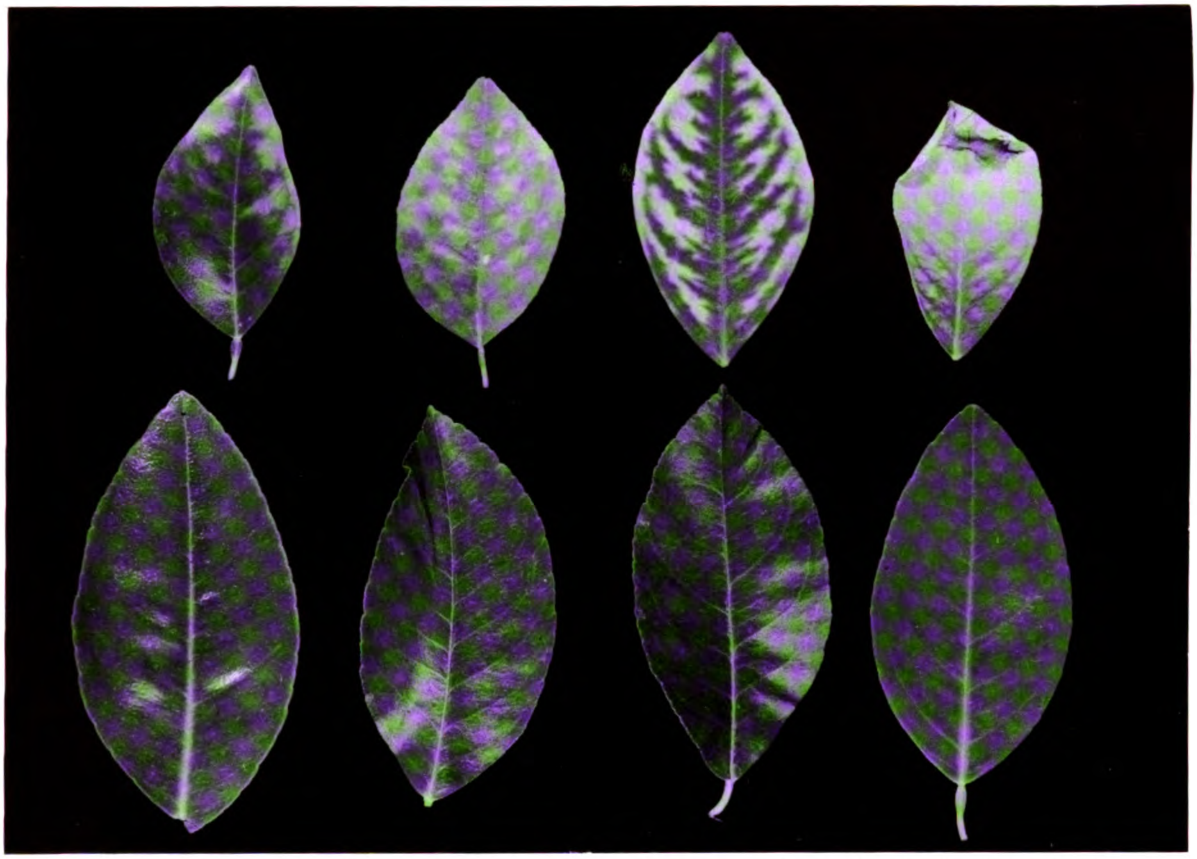

Fig. 3–11. Leaf chlorosis and necrosis patterns produced on orange leaves by fumigation with hydrogen fluoride (top), as compared with size and appearance of similar age leaves from control trees (bottom). Fluorine injury reduces leaf size and first produces a slight marginal yellowing, which is more marked toward the leaf apex. As the injury progresses, patterns resembling manganese deficiency and boron excess may appear. There is also tip and marginal necrosis.

citrus by Kaudy *et al.* (1954, 1955). Values up to 211 ppm of fluorine in the dry matter of citrus leaves were found in orchards near the steel plant, as compared with values of less than 10 ppm in areas away from industrial centers. Investigations by Brewer *et al.* (1959, 1960*a*, 1960*b*) under controlled conditions were immediately begun to assess the importance of fluorine to citrus performance. Meanwhile, in Florida, the investigation of a type of leaf chlorosis occurring on citrus trees in the vicinity of phosphate manufacturing plants by Wander and McBride (1956) disclosed that the cause was fluorine air pollution, and leaf values up to 370 ppm of fluorine in the dry matter of the leaves were found. In areas away from these plants, the fluorine content ranged from 12 to 30 ppm. Spray applications of $0.1N$ hydrogen fluoride or fluosilicic acid produced a similar type of chlorosis.

Actual field injury of citrus from excess fluorine has been reported only in Florida and California, and in both areas it is from atmospheric pollution. This is true for plants generally. Soil fluorine (occurring naturally or resulting from contamination by waters, fertilizers, or the atmosphere) is relatively insoluble and therefore not appreciably absorbed by plants.

The main sources of fluorine air contamination are: (1) aluminum reduction plants, (2) iron and nonferrous smelting plants, (3) ceramic industries, and (4) phosphate fertilizer plants. Naturally occurring fluorine or the fluorine in cryolite added in smelting and reduction operations is driven off by high temperatures; in the case of phosphate rock, treatment with acid releases fluorine contaminants. The chief volatilization products are hydrogen fluoride and fluosilicic acid.

The amounts of fluorine in the air, based on limited numbers of air analyses, vary in industrial areas from 0.1 up to 147 parts per billion (ppb), depending on the industry in question and proximity to the industry.

Plants vary enormously in their sensitivity to fluorine. Some sensitive plants (such as gladiolus and apricot) may be injured when the atmosphere contains less than 1 ppb of this element. Plant foliage generally tends to accumulate fluorine both as a surface contaminant and internally, but there are great differences in tolerance for reasons not yet understood.

Citrus is quite tolerant to fluorine, as compared with sensitive plants such as gladiolus, apricot, peach, some grape varieties, and Ponderosa pine. But there are also differences in citrus species and variety susceptibility.

Symptoms of Fluorine Excess.—Aside from a type of chlorosis that closely resembles the excess boron mottling described by Wander and McBride (1956) and shown in fig. 3–11, all that is known of the growth and symptomatology effects of excess fluorine has emerged from controlled nutritional and fumigation experiments.

Table 3–20

FLUORINE CONTENT OF MATURE NAVEL ORANGE TREES GROWN IN SOLUTION CULTURES WITH AND WITHOUT FLUORINE PRESENT IN THE NUTRIENT SOLUTION

	Fluorine Content in Dry Matter (Ppm)	
Tissue Analyzed	25 Ppm Fluorine Added*	No Fluorine Added†
Spring-flush leaves (1-year-old)	34.0	5.5
Branches (whole cross-sections):		
Large (over 1 inch)	5.5	2.0
Intermediate (¼ to 1 inch)	6.0	2.5
Twigs	10.0	5.0
Trunk:		
Old xylem	3.0	1.0
New xylem	3.5	2.0
Phloem and bark	8.3	4.0
Whole cross-section	7.0	3.0
Roots:		
Large roots, whole	67.0	6.0
Center wood (old xylem)	9.0	13.0
Intermediate wood (new xylem)	13.0	4.0
Epidermis	641.0	3.0
Small roots	20,000.0	46.0
Fruit:		
Rind	1.70‡	0.27‡
Pulp, juice	0.14‡	0.09‡

* Average of two trees.
† Average of four trees.
‡ Fresh weight.

In a water-culture experiment with four-year-old navel orange trees growing outdoors, Brewer *et al.* (1959) found that the addition of 100 ppm of fluorine to the aerated solutions resulted in leaf wilt within 24 hours. This was followed by complete defoliation. Another group of trees, given 25 ppm of fluorine in the nutrient solution, was grown along with control trees for 18 months. No specific leaf symptoms developed, but, compared with the controls, growth was reduced, spring leaf drop was more severe, leaf sizes were smaller, and the trees eventually became quite weak and sparsely foliated. Leaf fluorine in spring-cycle leaves collected over a period of months showed values up to about 60 ppm (dry-weight basis), compared with less than 10 ppm in the leaves from control trees. At the conclusion of the experiment, samples of leaves, branches, trunk, roots, and fruit were analyzed from both sets of trees. The results are reproduced in table 3–20. Some elevation of fluorine occurred in all parts of the tree, but that in the leaves and small roots was greatest. No doubt the very high values in fine roots were due, in part, to surface contamination of insoluble calcium fluoride.

In a sand-culture experiment with lemon cuttings (Haas and Brusca, 1955*a*), 400 ppm of fluoride produced tip burn, chlorotic leaf patterns resembling manganese and iron deficiencies, reduced leaf sizes, and reduced growth. No data on plant composition were given.

Fumigation of various citrus varieties under controlled greenhouse conditions by Brewer *et al.* (1960*a*, 1960*b*), using hydrogen fluoride gas at concentrations in the air varying from 2 to 12 ppb, reduced growth rate and leaf sizes and produced various types of leaf injury. The injury often began with marginal yellowing, tip and marginal burning, and chlorotic patterns resembling manganese deficiency and boron excess (see fig. 3–11). Injured leaves dropped prematurely, and this was frequently followed by multiple new weak growth emerging from axillary buds. Leaves showing the burn and chlorosis (fig. 3–11) usually contained more than 200 ppm of fluorine in the dry matter. Thomas (1961) noted that with many plants that fluoride absorbed by leaves is translocated to the margins and tips of leaves with gradients of 2:1 to 100:1 between the concentrations in the tips and margins as compared to that of the rest of the leaf. He stated that appreciable translocation from leaves to other parts of the plant does not occur.

Brewer *et al.* (1960*a*) noted species and va-

Table 3–21

PRINCIPAL FLUORINE EXCESS EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Fluorine Excess Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. First effects are reduction in leaf size and general loss of chlorophyll, especially at the margins of leaves and between veins.	1. Boron excess and manganese and zinc deficiencies produce somewhat similar leaf patterns.
2. Marginal tissues of leaves continue to decrease in chlorophyll content until margins are completely bleached, particularly at leaf tips. Chlorosis spreads inward toward the midrib.	2. Salt injury sometimes produces somewhat similar effects.
3. Many leaves develop typical manganese deficiency patterns, but with more mottling at the leaf apices.	3. Manganese deficiency. There is some indication that fluorine reduces manganese content of leaf tissues and therefore the chlorosis may in fact be a fluoride-induced manganese deficiency.
4. Necrosis of leaf apices may result from exposure to high fluorine concentrations or sudden moisture stresses.	4. Boron toxicity symptoms are very similar except that brown, pustular spots or flecking are usually found on the undersides of older leaves injured by boron.
Fruiting and Fruit Characteristics	
1. No apparent influence on fruit quality.	1. Many mild nutritional disturbances produce no effect on fruit quality.
2. Prolonged exposure to fluorine tends to reduce fruit set and thereby yields. Size of fruit may be increased due to small crop.	2. Sizes and yields are reduced by many other factors.
Tissue Analysis Values	
1. Threshold leaf analysis values for toxicity, as determined by greenhouse experiments using hydrogen fluoride (expressed as ppm fluorine in the dry matter) are as follows: Lisbon and Eureka lemons, 75 ppm; Dancy mandarin, 75 to 100 ppm; Valencia and navel oranges, 100 to 125 ppm; Marsh and pink grapefruit, 125 to 150 ppm; and Temple orange, 150 to 250 ppm.	1. None known at present.

riety differences in tolerance to fluoride, lemon and grapefruit species being somewhat more sensitive than orange species.

A summary of foliage, growth, fruit, and composition effects of fluorine on citrus is presented in table 3–21.

Control of Fluorine Excess.—From the available evidence, it appears that fluorine injury to citrus (and most plants, for that matter) is likely to occur only from air pollution and only when citrus is located within a radius of ten to twenty-five miles from air pollution sources.

With some plants, lime dusts and sprays have given protection against damage from fluorine air pollution. In the case of citrus, insufficient information is available as yet as to the effectiveness of lime.

In soils, the only likely circumstance where excess soluble fluorine would occur is under acid conditions. Therefore, the simple solution would be to neutralize the acidity with limestone.

IRON (Fe)

Iron Deficiency

The essentiality of iron for plants has been known for over one hundred years. Gris (1843) is said to have been the first to identify lime-induced chlorosis as an iron problem. He was able to green grape leaves by the use of iron sprays.

A number of reviews have been written dealing with one or another aspect of the occurrence, nature, cause, and control of iron deficiency. The reader is referred to the following works for a broader discussion of the subject than is possible here: Brown (1956, 1961); Brown, Holmes, and Tiffin (1959); Bear (1957); Thorne, Wann, and Robinson (1951); Wallace and Lunt (1960); Wallace (1962); Nicholas (1961); and Evans (1959).

The distinction between various types of citrus leaf chlorosis caused by lack of manganese, zinc, iron, and magnesium did not become clear until the range of patterns of these disorders was delineated by Camp and Fudge (1939) and Chapman and Kelley (1943). The visual range of foliage, growth, and fruit effects of both acute and mild deficiencies is now well known, and much has been learned about the multiple causes of this disorder.

While iron chlorosis is less prevalent and more spotty in its incidence than zinc deficiency, it is reasonably widespread throughout the world and a problem of great commercial importance.

Symptoms of Iron Deficiency.—The predominant leaf pattern produced by the lack of iron (most pronounced on terminal shoots) can best be described as a network of fine green veins against a lighter-colored background; symptoms range in severity from a barely distinguishable venation to one in which the leaf becomes almost totally yellow or ivory-colored, with perhaps just a tinge of green in the basal part of the midrib. At some stages (in young growth particularly), it is difficult to differentiate the symptoms from manganese deficiency, and often both disorders occur in the same tree simultaneously. In such cases, one can best judge the situation by looking only at mature leaves. With iron deficiency, the network of green veins continues to be sharply delineated from the lighter-colored areas between the veins as the leaf matures, whereas with manganese and zinc deficiencies a fringe or band of green develops along the midrib and main veins, producing a more mottled appearance. As stated, in severe deficiency the leaves may appear almost completely cream-, ivory-, or yellow-colored, and sometimes they become tinged with red or brown; acute zinc deficiency can produce comparable symptoms, though it is fairly common in this case to see small green dots in mesophyll areas.

Photographs illustrating typical leaf patterns on lemon and orange leaves are reproduced in figures 3–12, 3–13, and 3–14. When one is in doubt about the diagnosis, the easiest way to confirm it is to paint or dip individual leaves with a solution of iron sulfate and then tag them for identification. A 6.5 per cent ferrous sulfate ($FeSO_4 \cdot 7H_2O$) solution can be used. This solution contains about 1,000 ppm of iron. If the condition is due to a lack of iron, partial greening of the leaf

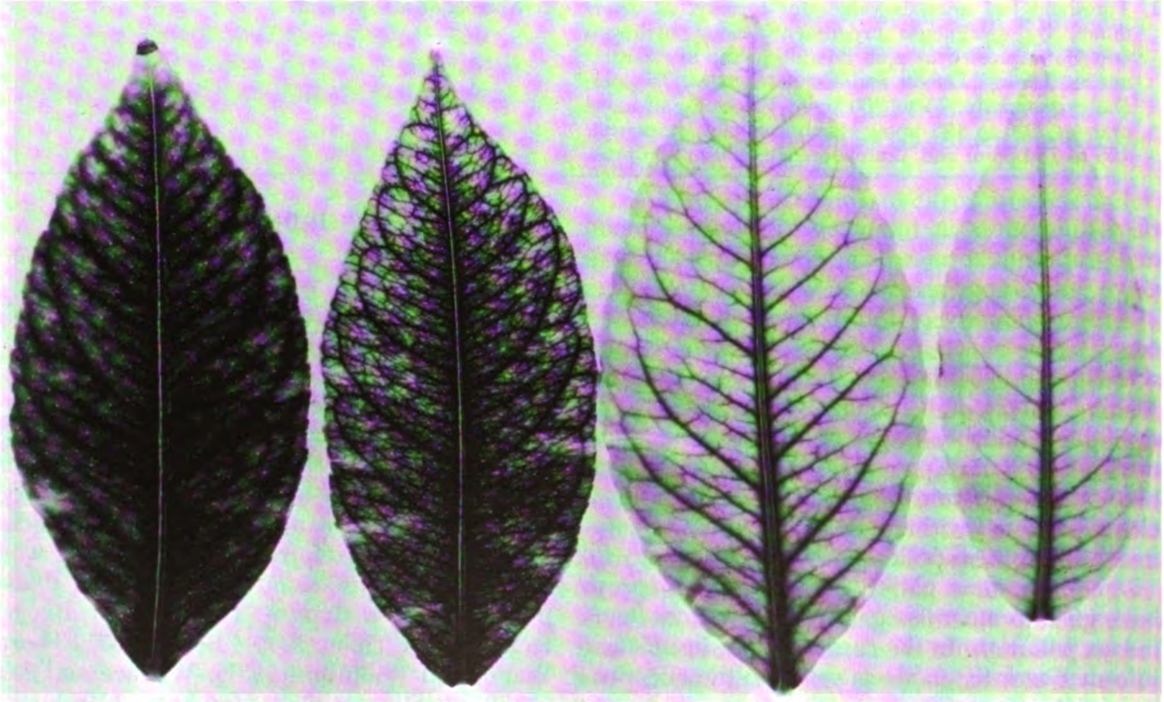

Fig. 3–12. Mature lemon leaves showing (left to right) progressive stages of iron deficiency. In the very acute stage, leaf may fade to almost pure cream-yellow, with only a trace of green in the midrib. The most common symptom is a fine network of green veins against a lighter-colored background.

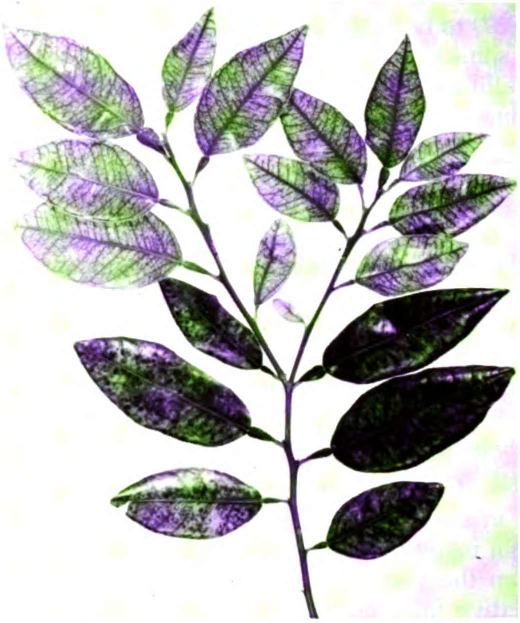

Fig. 3–13. Orange twig showing iron chlorosis of new-cycle growth, which emerged following iron sulfate spray treatment on older flush of growth. There was partial recovery of the old cycle. This indicates that there is no apparent benefit to succeeding growth cycles, even though iron sprays bring about some recovery of treated leaves.

or a speckling of green spots will appear. It usually requires one to several weeks for this to occur, depending on the time of year and the leaf age (see figs. 3–13 and 3–14).

Leaf analyses for total iron may not always be conclusive, though leaf levels of 10 to 35 ppm of iron in the dry matter, associated with iron chlorosis, would constitute fairly conclusive evidence that the condition was due to lack of iron. According to Wallihan (1966), the very earliest stages of iron deficiency may merely reduce leaf size and not produce a chlorosis.

Acute iron deficiency results in a progressive dieback of trees, giving them a brushy, open appearance. Often one limb is affected more than others, and the tops are more affected than the lower parts. Leaves do not reach normal size, and there is premature abscission of affected foliage. Fruit production is curtailed, and, even where the condition is mild, less mature fruits are to be found on mildly iron-deficient terminals than on healthy green ones.

Fruit quality does not appear to be severely affected. Generally, the fruit on trees affected with this disorder is smaller and tends to be smoother than fruit from healthy green trees. Total solids are reduced, but acidity increases somewhat. Mature oranges may be less highly colored.

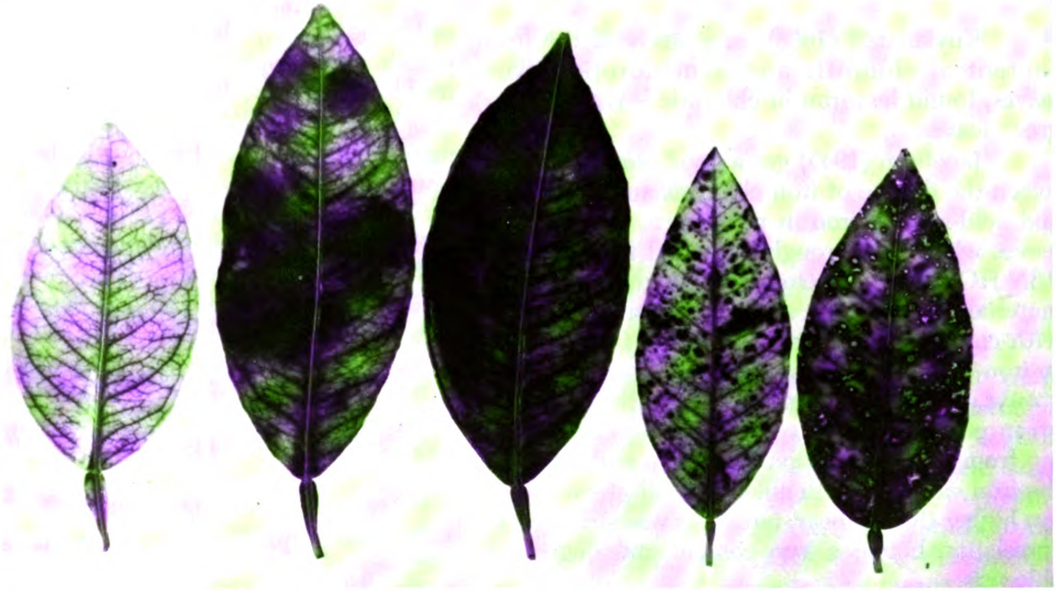

Fig. 3–14. Orange leaves showing effects of iron sprays with and without spreader. Leaf at left was unsprayed; next two leaves were sprayed with iron sulfate containing a spreader; and the two leaves on the right were sprayed with iron sulfate without spreader. Note the necrotic burn from the spray on the last leaf at the right. The spreader reduced necrotic spotting, but it did not prevent leaf abscission. (Photo courtesy of Guest and Chapman, 1949.)

Leaf Analysis Values.—As far as leaf composition is concerned, there is good evidence with citrus, if leaves are thoroughly cleaned with detergent and/or weak acid (e.g., 0.3*N* HCl), that a fairly good correlation exists between iron content and degree of chlorosis. Smith, Reuther, and Specht (1950) found that mature citrus leaves, thoroughly cleaned with a detergent solution (sodium lauryl sulfate), showed the following values on a dry matter basis:

Degree of Chlorosis	*Total Iron (Ppm)*
Nonchlorotic leaves	35 to 100
Mildly chlorotic leaves	18 to 30
Severely chlorotic leaves	10 to 15

Wallihan (1955) got a fairly good correlation between degree of chlorosis and iron content on orange, lemon, and grapefruit leaves. He washed each leaf carefully in a soap solution and then thoroughly rinsed it. The data were as follows:

Degree of Chlorosis	*Iron in Dry Matter (Ppm)*
No chlorosis	42 to 137
Moderate chlorosis	32 to 68
Severe chlorosis	24 to 59
Extreme chlorosis	16 to 33

Kuykendall (1955), in a study of the iron content of chlorotic and nonchlorotic citrus leaves, found less iron in chlorotic leaves than in green leaves.

Jacobson (1945) got a good correlation between the degree of iron chlorosis and both total and acid-soluble iron in pear, corn, and tobacco leaves when he thoroughly cleaned the leaves in 0.3*N* HCl. Iron extracted from dried, ground-leaf material by 1.0*N* HCl showed a good correlation with chlorophyll extracted from the fresh leaves. In iron-chlorotic pear leaves, the iron content was always under 40 ppm on a dry matter basis. Deep-green pear leaves showed total iron values ranging from 39.2 to 79.5 ppm. For corn and tobacco, the iron values in both chlorotic and green leaves ran higher than in pear, but there was still a good correlation between iron content and degree of chlorosis.

As stated, deficiency levels of iron in citrus leaves usually range from 10 to 40 ppm iron in the dry matter, though there is some overlapping. The leaves of some top-producing, nonchlorotic orchards were found by Bradford and Harding (1957) to be in the 30- to 40-ppm range; conversely, some iron-deficient citrus foliage was found by Wallihan (1955) to show as high as 68 ppm of iron.

It is well known that iron is much less mobile in green plants than any of the other essential elements, and there is considerable evidence to indicate that a variety of internal conditions, such as high phosphorus, low potassium, low magnesium, and probably other nutritional imbalances, can reduce iron availability. Thus, close correlations between iron levels in the leaf and degree of chlorosis are not to be expected.

A summary of the effects produced by iron deficiency on citrus is given in table 3–22.

Causes of Iron Deficiency.—Iron deficiency in citrus (and in plants generally) can result from many causes. This appears to stem, in part, from the very low solubility of iron in soils, its relative immobility in plants, and the fact that luxury consumption and/or translocation and reuse does not go on to the same degree as with many of the other nutrients. Iron deficiency may also result from the multiple roles of iron in plant metabolism and the probability that unbalanced nutritional conditions of many kinds can interfere with one or another of these functions.

Under slightly acid, neutral, and alkaline soil conditions, it is certain that a part of the plant's iron supply comes from numerous localized root-soil particle zone contacts.

At pH 4, Wallace and Lunt (1960) estimated the solubility of ferrous hydroxide as 0.003 ppm of iron. Data supplied to the author by Dr. H. Bohn, Postdoctoral Fellow, Department of Soils and Plant Nutrition, University of California at Riverside, are of interest. This investigator analyzed soil solutions which had been equilibrated with 0.01*M* calcium chloride for four months. Twenty grams of soil were placed in one liter of 0.01*M* calcium chloride and saturated with air for four months prior to analysis. The iron content of the solution phase at various soil suspension pH values was as follows:

Soil Suspension (pH)	*Iron in Solution (Ppm)*
3.88	0.04
4.43	0.007
4.70	0.002
4.78	0.002
4.78	0.003
5.14	0.003

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Table 3–22

PRINCIPAL IRON DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Iron Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Terminal leaves show a typical venation, best described as a green network of veins on a lighter-colored background; many stages of this can be seen (see figs. 3–12 and 3–13). Where chlorosis is severe, leaves may become entirely yellow with only a tinge of green in the midribs. Affected leaves are usually smaller and thinner, but this tendency is not nearly as marked as where zinc is deficient. Acutely iron-deficient leaves may be tinged with orange or bronze coloring and may show brown discolorations on the most exposed parts of the leaves.	1. Acute stages of manganese deficiency produce leaf patterns, especially on lemons, similar to those of iron deficiency. Often both iron and manganese deficiencies occur simultaneously on the same tree—one branch may show foliage which is primarily iron-deficient and on another the deficiency may be primarily a lack of manganese. However, general over-all appraisal of foliage will usually reveal whether the deficiency patterns are essentially those of iron or manganese deficiency.
2. Frequently, new terminal growth will show typical venation, as described above under (1.), with older foliage remaining completely green.	2. Terminal growth is more affected than old growth in both manganese and zinc deficiencies.
3. Where the condition is moderate to severe, one branch may be more affected than another. Dieback of severely affected twigs and limbs is common.	3. In magnesium deficiency, it is common to find one branch more severely affected than another, with ensuing dieback.
4. Affected leaves are usually shed prematurely.	4. Premature leaf drop is common with many other conditions.
5. Deficiency patterns are often more common in winter than in summer. Regreening may cause partial or complete disappearance of venation.	5. With manganese and zinc deficiencies, patterns are more prominent in the fall, winter, and spring, with greening in the summer.
6. Wallihan (1965) states that in early stages chlorosis may not develop and the only effect is decreased leaf size.	6. In many disorders, the first noticeable effect is reduction of leaf size.
Fruiting and Fruit Characteristics	
1. Yields and fruit set are less; often there are no fruit sets at all on moderately to severely affected terminals.	1. Yield reductions occur from many other deficiencies.
2. Fruits tend to be smaller, but they are smooth; no special adverse internal characteristics are seen until the deficiency becomes acute. In this case, total solids are less and acidity is higher; also, the fruit may appear more lemon-colored than is normal. See Pratt (1958) for Florida; Embleton and others for California (unpublished observations).	2. Many other nutritional deficiencies result in small fruits.
Tissue Analysis Values	
1. Total iron in dry matter of leaves showing various stages of iron deficiency runs from 10 to 68 ppm of iron, as compared with a range of 40 to 150 ppm in green leaves. There is thus some overlapping.	1. None known.

Oertli and Jacobson (1960) found that plants could not get enough iron from maintained solution concentrations comparable to those existing in soils.

The low solubility of many iron compounds may also be a factor in the immobility of iron within leaves and the ease with which its transport in the vascular system is upset. The extent to which naturally occurring organic chelates of iron are present in soils and in plants and their relative importance in iron nutrition, generally, is little known at present.

The ability of plants to extract iron from highly insoluble sources was first demonstrated by Eaton (1936), who found that 0.1 per cent of ground magnetite (incorporated in the acid sand cultures) made the addition of soluble iron compounds unnecessary. In a continuing study using magnetite, Chapman (1939) showed that orange seedlings growing in a sand-magnetite mixture were able to extract iron from magnetite through a root-iron particle contact mechanism, and that additions of calcium carbonate to the sand-magnetite culture markedly reduced iron extraction.

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By increasing the magnetite supply, the effect of calcium carbonate could be overcome. The sand cultures in this experiment were periodically flushed with a nutrient solution from a common reservoir, using a time-clock-controlled, compressed-air pumping arrangement. It was found that frequent or continuous flushing of the calcium carbonate-magnetite-containing sand cultures brought on iron chlorosis and an increase in alkalinity of the circulating nutrient solution (from pH 7.3 to 8.2). This was the result of the continuous aeration of the circulating nutrient solution by the compressed-air-ejector-type pumping arrangement, and the resulting removal of carbon dioxide produced by the plant roots. Iron solubility in the nutrient medium was reduced from 0.01 ppm iron at pH 5.9 to less than 0.005 ppm at pH 8.1. The pH change in the periodically circulating culture solution no doubt influenced the pH in the intimate zones of root-iron particle contact and prevented the roots from securing adequate iron from the magnetite. Guest (1944) confirmed some of these findings.

In somewhat parallel experiments, using alfalfa as a test plant, Jenny (1961) and his co-workers, Glauser and Jenny (1960*a*, 1960*b*), Grunes and Jenny (1960), and Charley and Jenny (1961), confirmed and extended some of the aforementioned findings and showed that:

1. In calcareous, alkaline media the rate of iron uptake by young alfalfa plants is directly proportional to the number of solid, iron oxide grains which touch the root surface.
2. Hydrogen-ion exchangers (e.g., amberlite and amberplex membranes and roots) readily decompose iron oxide particles by contact interaction.
3. The iron ions acquired at the outer surface of amberplex membranes and root plugs migrate through these porous bodies by exchange diffusion.

Wynd (1951) found that wheat plants could extract iron through a root-contact mechanism from a glass frit containing 5 per cent ferric oxide.

The presence of considerable magnetite and other iron compounds in soils and their low solubility, on the one hand, together with the prevalence of iron chlorosis on some calcareous soils and not on others, the seasonal fluctuations in iron chlorosis, effects of excess moisture, low temperatures, and lack of oxygen, all fit in with the idea that in many situations root-soil particle contact exchange is one of the important mechanisms by which plants secure needed iron from soils.

A brief discussion of the factors known to produce or aggravate iron deficiency in citrus and other plants follows:

Calcareous soils (lime-induced chlorosis).—Excess lime often produces severe iron chlorosis in citrus and most other plants. While many explanations have been advanced, the most reasonable is the low solubility of iron under the pH conditions which calcium carbonate produces in soils and the buffering effect of calcium carbonate. The calcium bicarbonate produced in such soils may possibly have some effect on both the iron absorption and its mobility within the plant. But this explanation does not appear entirely plausible, since iron-deficient leaves usually show lower-than-normal calcium levels (and higher potassium). Also, Chapman (1939) and Glauser and Jenny (1960*a*) found that the effects of calcium carbonate could be overcome by the addition of more magnetite or iron-coated sand grain to the rooting medium. The fact that not all soils containing free lime produce iron chlorosis can be explained by any one or a combination of conditions, including the amount, form, and particle sizes of the lime and iron compounds present; variables in the organic iron content of the soil; and aeration, temperature, moisture, and biological-activity fluctuations.

Overmoist soil conditions and aeration.—There is abundant field and pot-culture evidence that persistent overmoist soil conditions will produce iron deficiency in citrus and many other plants. In fact, this is one of the most common causes of, or contributing factors to, iron chlorosis of citrus in California. Until the advent of chelates, the most effective way to correct iron chlorosis of citrus was to modify irrigation practices by various means, such as alternate middle irrigation, changing irrigation frequency, and the guidance of irrigation by the use of tensiometers. All of these means were designed to reduce or obviate persistent overmoist conditions. The simplest explanation of the effects of too much moisture is lack of adequate aeration. Wallihan *et al.* (1961) have recently shown in pot experiments with porous soil that, by reducing the oxygen content of the atmosphere over the surface of the soil from 18 to 21 per cent to 2 to 6.2 per cent, the growth and iron content of leaves was reduced from 64 to 37 ppm of iron, using a soil of pH 6.2, and from 48 to 27 ppm of iron where calcium carbonate had

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been added to the soil. In the presence of calcium carbonate and low oxygen, the leaves showed iron chlorosis. Low oxygen, of course, will reduce respiration rate and production of carbon dioxide, and thus also reduce the degree of acidity and its solubilizing influence on iron in solid-phase forms.

Higher moisture in soils also will slightly increase hydrolysis of base-carrying minerals and thus slightly increase the alkalinity of the soil. This increase may be just enough in some cases to make the difference between iron sufficiency and insufficiency.

Under high moisture conditions, more manganese is solubilized, and this may be a further contributing cause to the prevention of iron absorption by, or translocation out of, the root. Changes in soil organism populations and activities may also be brought about by high moisture conditions.

Bicarbonate.—There is substantial evidence that the bicarbonate ion, *if present in sufficent amounts,* can bring on or aggravate iron deficiency. In sand cultures containing 1 per cent powdered magnetite as a source of iron and periodically flushed with a complete nutrient solution from large reservoirs, Pearson and Chapman (1964, unpublished data) found that 30 meq of sodium bicarbonate added to the nutrient solution produced severe iron chlorosis of citrus plants, whereas comparable amounts of sodium chloride, sodium sulfate, and sodium nitrate did *not* cause iron chlorosis. The pH of the high sodium chloride, sulfate, and nitrate cultures varied between pH 5.5 and 6, but those containing the sodium bicarbonate ranged from pH 8.2 to 8.5. No doubt, the higher pH and buffering capacity of this solution made it impossible for the citrus roots to create sufficiently acid root-iron particle contact zones to extract needed iron. The bicarbonate, of course, as will be mentioned later, may have exerted other effects.

In five-hour absorption period experiments with radioactive iron (Fe^{59}), Wallihan (1961) showed that 10 meq of sodium bicarbonate added to a nutrient solution (complete except for phosphate), prevented radioactive iron from being taken up into the stems and leaves of sweet orange seedlings and greatly reduced the radioactive iron found in the roots. The control cultures contained 10 meq of sodium sulfate.

All irrigation waters carry some bicarbonate. Depending on the relative amounts of sodium, calcium, and magnesium in such waters, there may be deposition of both sodium bicarbonate and calcium and magnesium carbonate in irrigated soils. Acid or neutral soils will become alkaline, and iron-bearing minerals may be coated with insoluble carbonate. Broadbent and Chapman (1950) found in a 15-year outdoor lysimeter experiment that the application of a rather low-salt irrigation water (347 ppm of total solids), of which 2.94 meq (181 ppm) were bicarbonate, changed soil pH (paste) from a value of about 6.8 to 8.5 and deposited several tons of calcium carbonate per acre. The rate of irrigation water usage was about 26 acre-inches per year. Harley and Lindner (1945) found that the use of well waters high in bicarbonate (210 to 360 ppm) led to lime deposition in soils and on plant roots and produced iron chlorosis in apple and pear orchards.

Bicarbonate may raise the pH of plant sap (Wallace, 1962) and thus decrease iron solubility. Miller and Evans (1956) showed that the cytochrome oxidase activity of root preparations was decreased more by bicarbonate than by chloride, sulfate, nitrate, or phosphate ions. Many other investigators, working with a variety of plants, have noted that bicarbonate in some manner adversely affects iron absorption and/or translocation or utilization in the plant. (For a more complete review of other investigations on this subject, the reader is referred to the citations mentioned on page 161).

Nutrient imbalance.—Excesses and deficiencies of both macroelements and microelements can cause iron chlorosis in citrus and other plants. A brief discussion, element by element, follows:

Potassium.—In outdoor water cultures with bearing citrus trees, the author and his colleagues have repeatedly noted persistent iron chlorosis patterns on trees acutely deficient in potassium. Iron sprays applied to these leaves caused the familiar partial greening, and leaf analyses (Chapman, Brown, and Rayner, 1947) showed an iron content of 35 ppm in affected leaves, as compared with 90 ppm in similar healthy trees. We have also noted iron chlorosis in trees produced under low calcium and high potassium conditions.

Phosphorus.—The author has found, under neutral or alkaline nutrient-culture conditions, that high phosphorus can sometimes produce iron chlorosis. Many investigators (Olsen, 1935; Bolle-Jones, 1955; Biddulph and Woodbridge, 1952; and Brown, Holmes, and Specht, 1955), working with

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other plants, have obtained similar results. It appears that this effect is more likely due to iron precipitation within the plant than to interference with absorption by the root and may depend upon associated conditions within the plant; e.g., calcium, copper, pH, and the respective levels of iron and phosphate.

There is considerable doubt about the field importance of excess phosphorus, however, as copper and zinc absorption or utilization becomes limiting first. In pot-culture studies with nine California soils, Bingham *et al.* (1958) found that, in soils varying from pH 4.4 to 7.4, application of monocalcium phosphate (up to 1,800 pounds of phosphorus per acre) did not reduce iron absorption, but markedly reduced copper uptake.

Calcium.—Aside from the lime effect mentioned, high concentrations of calcium ions are frequently reported as producing iron chlorosis in some plants.

Magnesium.—In an outdoor sand-culture experiment, where prolonged acute magnesium deficiency was produced in Valencia orange trees, the author noted that secondary iron chlorosis began to appear on new foliage. This condition could be corrected by iron sprays. In other experiments (Chapman, 1944, unpublished data) with high magnesium, the addition of 100 meq of magnesium nitrate to lemon cuttings in nutrient cultures produced severe iron chlorosis. The roots of these cuttings became thickened and stubby.

Zinc.—In the course of nutritional work with citrus, Chapman *et al.* (1940) tried to correct zinc deficiency in Valencia orange cuttings grown in sand cultures by the addition of a few grams of zinc dust or zinc oxide. Instead of correcting the zinc deficiency, marked iron chlorosis developed. Further research confirmed that excess zinc in the nutrient medium would cause iron chlorosis. Analyses of leaves, stems, and roots showed that the chlorosis was due to failure of sufficient iron to reach the leaves and a probable tie-up of iron in the roots or the cortex. Hewitt (1948) produced iron chlorosis in sugar beets when 0.5 to 1 meq of zinc was added to the nutrient medium. Also, Hunter and Vergnano (1953), Millikan (1947*b*), and others have produced iron deficiency from the addition of zinc under controlled culture conditions.

Copper.—In our early work with zinc (Chapman *et al.*, 1940), it was found that excess copper would sometimes produce iron chlorosis in sand cultures. In Florida, as mentioned earlier in the section on copper, Reuther and Smith (1953) and Smith and Specht (1953) found that copper accumulations in soil from the use of Bordeaux sprays and soil applications of bluestone were responsible for iron deficiency under acid soil conditions. This subject was extensively investigated. The findings were that affected citrus showed low iron content in chlorotic foliage, marked reduction in vigor and yields, dieback of trees, and sparse, stubby roots in the zone where copper had accumulated. Compared with total copper levels of 5 ppm or less in soils where Bordeaux had never been used, values of 50 to 200 ppm copper were found in the soils where such citrus troubles occurred.

Other workers (Willis and Piland, 1936; Hunter and Vergnano, 1953; and Hewitt, 1951) have shown with other plants that excess copper can produce iron chlorosis. Brown *et al.* (1955) found that various copper-phosphorus combinations would cause iron chlorosis in some varieties of soybean.

Other heavy metals.—Other metals—notably nickel, cadmium, cobalt, chromium, molybdenum, and manganese—have been shown capable of producing iron chlorosis in various plants (Millikan, 1947*b*; Hewitt, 1948, 1951, 1953; DeKock, 1956; Hunter and Vergnano, 1953; and Brown, 1956). The effectiveness of these metals varies, and there are great differences between plants in their susceptibility to metal-induced iron chlorosis. Smith and Specht (1953) found with citrus that manganese could induce iron chlorosis.

While the mechanisms of these effects are not exactly known, the idea has been put forward that certain heavy metals displace or compete with iron in chelate-protein centers within the roots, and the order of effectiveness in producing iron deficiency is related to the relative stability of the various metal chelates (see Hewitt, 1951; Brown, 1956, 1963; and Wallace, 1962).

Low soil temperatures.—In California, it is a common observation that iron chlorosis of citrus (especially lemons) is aggravated in the wintertime when soil temperatures under 55°F occur and persist for considerable periods. In an outdoor water-culture experiment with bearing lemon trees currently under way, the author has noted that in spite of the presence of ample iron in the culture solution, iron chlorosis developed on the winter growth cycle. Leaves painted with iron sulfate became green, thus demonstrating that the chlorosis was due to lack of iron and failure of the roots to absorb or translocate sufficient iron to meet the needs of the leaves. In such cases, both in the field and in controlled cultures, the chlorosis cleared when the root zone warmed up.

Similar observations have been made with gardenias (Baudenistel, 1957). With this plant, FeEDTA soil applications greatly reduced the chlorosis.

Soil organisms.—It has been a common observation that young citrus plantings on prefumigated soil often suffer less iron, zinc, and manganese deficiencies than comparable plantings on nonfumigated soil. This may be due to better root growth and feeding power if root-injuring nematodes and soil fungi are not present. Whatever the mechanism, it is clear that root-attacking soil organisms can cause or aggravate iron chlorosis. Conversely, soil organisms undoubtedly are important in making iron available to plants through the production of carbon dioxide and organic chelating agents.

Other factors.—In addition to the foregoing, other soil and environmental factors (such as high light intensity, high soil temperatures, viruses, and high nitrate nitrogen) have been mentioned as involved in the iron chlorosis problem with some plants under certain conditions.

Effect of Variety and Rootstock.—As with many other plants, there are differences in susceptibility of various citrus varieties to iron chlorosis, and rootstocks also play an important role.

Lemons, for example, are more prone to chlorosis (particularly that due to low soil temperatures) than are oranges and grapefruit. Oranges are more prone to iron chlorosis on trifoliate orange rootstock than on rough lemon rootstock. Many other plant species and varieties show great differences in ability to extract adequate iron from the soil.

Control of Iron Deficiency.—Until the spectacular correction of iron deficiency, reported by Stewart and Leonard (1952*a*), with an iron chelate FeEDTA (iron ethylenediamine tetraacetic acid) in Florida citrus, the use of iron sprays, tree-injection procedures, and massive soil applications of iron sulfate had been only partially successful. In irrigated areas, as previously mentioned, better control of soil moisture so as to avoid persistent overmoist conditions (achieved by decreased irrigation frequency, alternate middle irrigation, use of green manure crops, better surface drainage, and use of tensiometers to guide irrigation practices) had often been the most practical and effective means of reducing iron chlorosis in citrus.

The aforementioned results with iron chelates opened up a whole new chapter in iron chlorosis control, not only with citrus, but with plants in general.

It was soon found that though FeEDTA worked miraculously on the acid soils of Florida, it was not as effective on calcareous soils (Stewart and Leonard, 1957). This discovery has led to much research to develop more effective compounds for these latter soil types.

Of the many compounds tried, one described as EHPG or EDDHA-Fe^3, and chemically as sodium ferric ethylenediamine di-(*o*-hydroxyphenylacetic acid) or Chel-138, has proven to be among the best for neutral and calcareous soils. This compound contains 6 per cent metallic iron, and it is generally used at rates of ⅓ to ½ pound per mature tree on light sandy soils, and ½ to 1 pound per tree on heavy soils. The material is uniformly distributed over the soil surface beneath the canopy of the tree. Following application, the material is thoroughly watered into the soil. The treatment is applied in the spring, with another application in the summer, if required. EHPG has given good results at rates of 12 to 24 gm of iron per tree in both the sandy and heavier calcareous soils of Arizona. Kuykendall, Hilgeman, and Van Horn (1957) and Cooper (1957) found that 5 gm of iron in the form of EDDHA-Fe^3 and another iron compound RA-157-Fe were highly effective on a calcareous fine sandy loam (pH 7.9) with three-year-old Dancy tangerines on Cleopatra mandarin rootstock.

With twelve-year-old Redblush grapefruit trees, suffering from iron chlorosis associated with the virus disease cachexia and high soil salinity, 1 pound of EDDHA-Fe^3 per tree cleared up the iron chlorosis, and the trees remained green for ten months (Cooper and Peynado, 1959*a*).

Kochan (1962), working with severely chlorotic peach trees, secured good correction with EDDHA-Fe^3 on calcareous soils when using an injection method. He devised an injector from a conventional spray gun. A solution was prepared containing 1 pound of EDDHA-Fe^3 per 25 gallons of water. A spray rig developing 400 pounds of pressure was used to make twelve injections 24 inches deep in a radius 4.5 feet from the tree trunks. This treatment, applied on May 25, 1961, produced 93 per cent correction of iron chlorosis by June 20, 1961.

Under the acid sandy soil conditions of Florida, use of FeEDTA at rates of 20 gm of iron per tree has been very successful (Stewart and Leonard, 1957). Since this compound is water-soluble, leaches away, and in due course decomposes, soil applications need to be made every two to three years. The material has not proven especially toxic, and the aforementioned investigators found that it required up to 1,000 gm of iron as FeEDTA to produce toxic leaf symptoms. This material can be mixed with other fertilizers, though its effectiveness may be decreased some by phosphates and lime.

In comparisons with other chelates for acid soils, FeEDTA has given good performance. Root growth is increased by FeEDTA (Ford, Stewart, and Leonard, 1954).

In summary, control of iron chlorosis in citrus can now be achieved in most cases by one or more means, the particular method used depending on the cause or causes.

Better control of soil moisture will result in improvement where overirrigation is implicated; soil fumigation will often improve iron chlorosis by making for better root growth; leaching of soluble salts will help where salinity buildup, particularly soluble bicarbonate, is a part of the salinity picture; correction of other deficiencies where they occur will help; where excess copper, zinc, manganese, or other metals occur (especially under acid soil conditions), controlled liming to bring the contaminated soil up to pH 6 to 6.5 will help; and, finally, the various chelates already discussed are effective.

Iron Excess

Except for the leaf-injury effects noted by Stewart and Leonard (1957), when they added up to 1,000 gm of iron as FeEDTA to soils, and injury to leaves and fruit from sprays of iron sulfate and other iron compounds, there has been little investigation of excess iron effects on citrus.

In plant nutritional work with citrus, the author occasionally has seen a fine speckling of necrotic spots on leaves after the accidental addition of too much iron sulfate to the nutrient

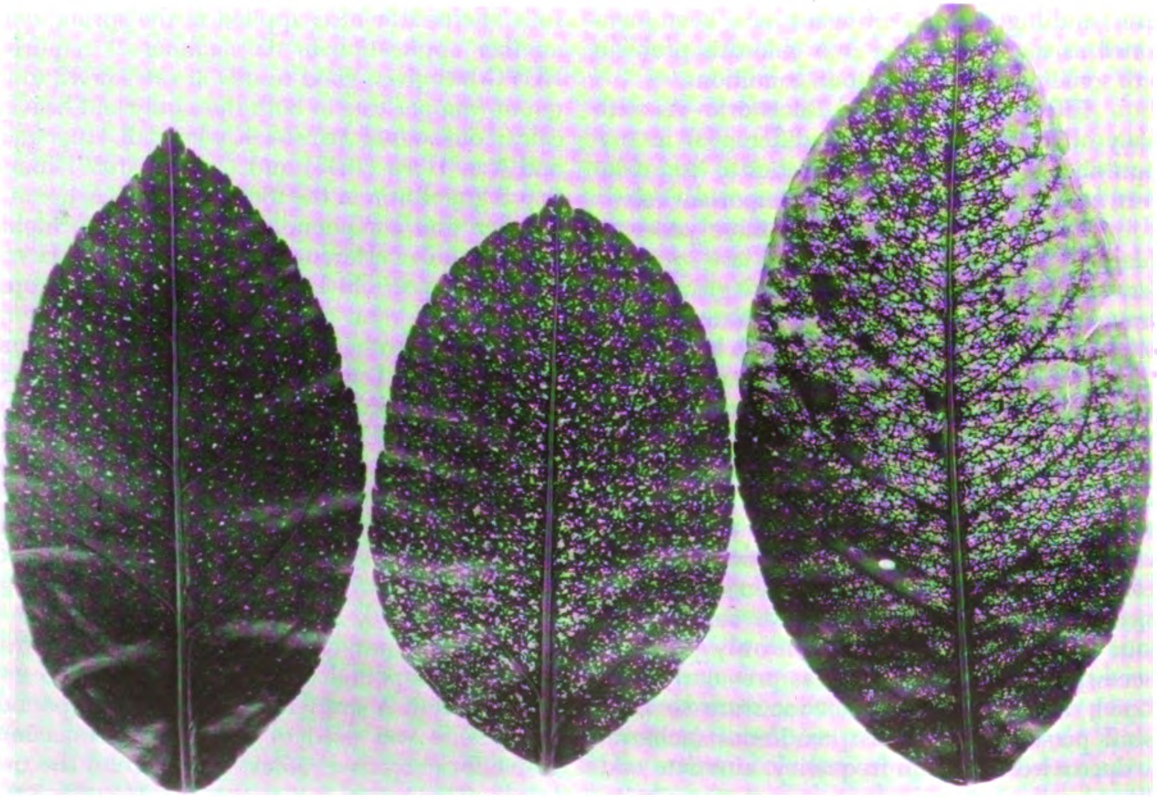

Fig. 3–15. Lemon leaves showing injury caused by adding too much iron sulfate to a nutrient solution.

solution (see fig. 3–15). He has also noted an occasional leaf drop on young orange trees in large 700-liter water cultures maintained at pH 4 to which 0.1 ppm of iron as iron sulfate had been added. Apparently when small plants with an actively absorbing root system are grown in large containers, enough iron can be absorbed from fairly dilute solutions to produce iron toxicity. Wallihan (1962, unpublished data) also noted this effect, and his analysis of injured leaves showed up to 239 ppm of iron in the dry matter.

Because of the generally low solubility of iron in soils under the pH ranges and the conditions of culture and soils in which citrus is grown, it is unlikely that iron toxicity will ever prove a practical problem except where excessive amounts of iron chelates are used.

In submerged rice soils, Ponnamperuma, Bradfield, and Peech (1955) found from 35.8 to 525 ppm of ferrous iron in percolates. Rice plants showed brown spots on older leaves, and the tips gradually took on a reddish-brown color, which spread toward the leaf base, especially along the edges. In time, young leaves began to show a brown speckling, and the older leaves dried up. Roots were coarse and brown. Symptoms were absent where drainage was good, submergence delayed, or there was a high initial concentration of nitrate in the soil. This is the only case known to the author of iron toxicity under field conditions.

LITHIUM (Li)

There is no evidence as yet that lithium is essential for citrus or green plants generally, but it is widely distributed in small amounts in rocks, soils, plants, and waters. The result of a recent survey of the lithium in 400 California irrigation waters by Bradford (1963) has shown that 25 per cent of them contain enough lithium (>0.05 ppm) to be potentially toxic to citrus and other lithium-sensitive plants.

Lithium Excess

Both stimulating and toxic effects of lithium have been noted by a number of workers in various crops. Some investigators have found that lithium in sprays and nutrient solutions has produced beneficial effects on certain plant diseases.

Bradford (1966) reviewed some of the literature on lithium distribution in soils and plants. Bradford's paper and the Chilean Nitrate Educational Bureau's *Bibliography of the Literature on the Minor Elements* (1948–55) provide a key to much of the lithium literature.

Haas (1929*a*) was the first to produce lithium-toxicity symptoms on citrus in controlled cultures. Later, Guest and Chapman (1946, unpublished data) applied various lithium salts to lemon trees in the field and produced marked leaf-injury symptoms (see fig. 3–16) and curiously a slight stimulation of tree growth. To explore further the possible stimulating and toxic effects of lithium under more controlled conditions, Aldrich, Vanselow, and Bradford (1951) set up pot-culture studies with citrus seedlings and produced the same leaf patterns as those illustrated in figure 3–16. It required only 2 ppm of lithium as lithium sulfate, added to the soil, to produce injury. In the field only 12.5 gm of lithium as lithium chloride per tree were required to produce toxicity symptoms in ten-year-old lemon trees. No growth-stimulating effects were noted.

Analyses of lemon leaves and fruits after lithium chloride had been applied in the field showed lithium concentrations as follows:

Lithium Applied to Soil in 18-foot Square Around Tree (Gm)	*Lithium in Dry Matter (Ppm)*		
	Mature Leaves	*Fruit Peel*	*Fruit Juice*
12.5	30.0	....	....
25.0	13.5	....	....
50.0	50.0	2.0	0.5

All of the leaves for which analyses are reported showed toxicity symptoms. Uninjured leaves from areas where lithium is not a problem usually show less than 1 ppm in the dry matter. Lithium, like boron, accumulates largely in the leaves of citrus trees and increases with leaf age.

Based on the experiments of Aldrich *et al.* (1951), lithium-toxicity symptoms were noted by the same authors on field citrus trees in Santa Barbara County, California. Samples of affected leaves showed the following values for lithium:

Kind of Citrus	*Lithium in Leaf Dry Matter (Ppm)*
Lemon	12
Grapefruit	14
Navel orange	23
Valencia orange	14

In the pot work by Aldrich *et al.*, analyses of various parts of sweet orange seedlings showed the following lithium concentrations.

	Lithium in Dry Matter (Ppm)					
Lithium Applied to Soil (Ppm)	*Young Leaves*	*Mature Leaves*	*Top Bark*	*Top Wood*	*Bark Root*	*Root Wood*
2.0	25.0	220.0	25.0	15.0	20.0	5.0
5.0	35.0	140.0	10.0	5.0	8.5	4.5

The toxicity was traced to lithium in the well waters used for irrigation. Four wells in the vicinity showed 0.075, 0.045, 0.055, and 0.08 ppm of lithium, respectively.

From the evidence available, it appears that soils derived from rocks and sediments high in ferro-magnesium minerals are likely to be higher in lithium than soils derived from granites and quartzite. According to Swaine (1955), total lithium in soils varies from about 8 to 400 ppm.

In the light of Bradford's (1963) water analysis survey, the possibility of lithium toxicity should be considered in evaluating citrus performance in irrigated areas, especially where waters and soils contain a higher-than-average sodium and boron content.

MAGNESIUM (Mg)

Magnesium Deficiency

The essentiality of magnesium for green plants has been known since about 1860. Magnesium was demonstrated to be a constituent of chlorophyll by Willstätter in 1906.

Reed and Haas (1924) were the first to produce and describe the effects of magnesium deficiency on young orange trees. Parbery (1935), working in New South Wales, Australia, described a type of leaf chlorosis on citrus with symptoms corresponding to those described by Reed and Haas (1924). Subsequently, Chapman and Kelley (1943) produced and described leaf symptoms of magnesium deficiency. In Florida, Bahrt (1934)

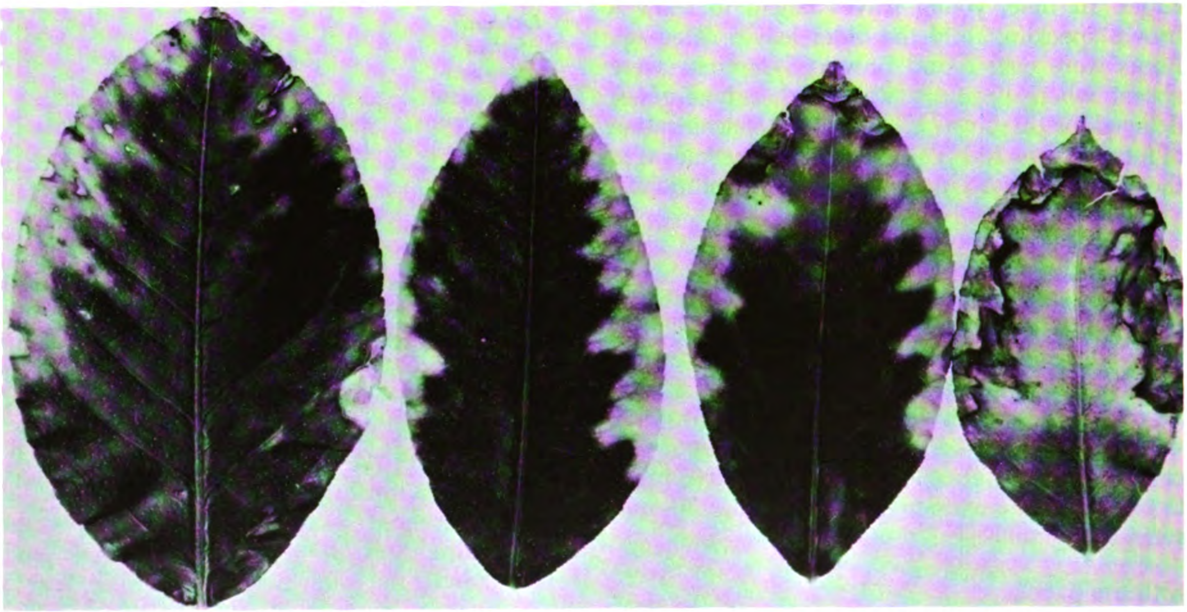

Fig. 3–16. Lithium excess effects on lemon leaves showing marginal chlorosis, followed by burning of leaf tips and edges. Oak-leaf-type patterns separating the green and yellowed areas are characteristic. Leaves so injured may contain 10 to 200 ppm of lithium in the dry matter. Normal levels are usually < 1 ppm.

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reported favorable citrus response to soil applications of magnesium, and further Florida work was soon reported by Tait (1936) and Bryan and DeBusk (1936). Later, Fudge (1938, 1939) and Camp and Fudge (1939) reported in more detail on magnesium deficiency in Florida. Good reviews dealing with magnesium nutrition in general are given by Embleton (1966), Uexküll and Kämpfer (1963), Hewitt (1963), and Jacob (1958).

Perhaps the most severe and widespread deficiency of magnesium in citrus occurred in Florida before the deficiency was recognized as such.

After identification and correction of widespread magnesium deficiency in Florida, this disorder was noticed in citrus areas all over the world. In California, mild magnesium deficiency was found rather widely, owing mainly to the accumulation of potassium in soils from the use of fertilizers and manures. The potassium accumulation in soils resulted in potassium-magnesium ratios unfavorable to magnesium absorption. Much the same pattern of occurrence has been noted in other citrus areas of the world (Oberholzer, 1941; Averna-Sacca, 1912; Raciti, 1956).

Symptoms of Magnesium Deficiency.—Leaf patterns and fruit effects of magnesium deficiency are shown in figures 3–17 and 3–18. A good description of the effects of magnesium deficiency was given by Camp *et al.* (1949) and is paraphrased in the following paragraphs.

Symptoms of magnesium deficiency occur in leaves at any season, but they commonly develop in the late summer or fall when the crop is maturing. The leaves are usually mature and normal in color up to the appearance of symptoms. Irregular yellow blotches start along the midribs of leaves that are close to fruit and eventually coalesce to form an irregular yellow band on each side of the midrib (see fig. 3–17). This area enlarges until only the tip and base of the leaf are green, with the base often showing a more or less inverted V-shaped area pointed on the midrib. This fading from green to yellow does not follow a fixed pattern, apparently being influenced by other nutritional factors and light, but in acute deficiencies the leaves eventually become entirely yellow. As soon as any considerable portion of the leaf becomes yellow, it may fall if conditions become unfavorable as a result of cold weather, toxic sprays, or other shocks to the tree. If conditions remain favorable, the yellowed leaf may stay on the tree for a long time. The defoliated twigs are weak and subject to fungus infection and may die by the following spring, necessitating a severe pruning of trees with a heavy crop.

Fudge (1939) showed that magnesium deficiency symptoms develop as a result of translocation of magnesium from the leaves to the growing fruit, although there may also be magnesium translocation from older leaves to young developing leaves on the same shoot. The mobility of magnesium in the tree differentiates it from iron, zinc, manganese, and copper. The symptoms due to deficiencies of these elements develop coincidentally with the new growth and not on older leaves which previously have been normal in appearance.

Varieties producing seedy fruit are more severely affected than varieties producing seedless fruit. In adjoining plots with the same treatment, seedy grapefruit may be almost defoliated while Marsh Seedless trees with an even heavier crop of fruit are only slightly yellow. A similar phenomenon is common in oranges, seedy varieties like the Pineapple being severely affected, while seedless Hamlins show only very slight yellowing. The yellowing is also related to the extent of fruiting. Heavily fruited limbs develop extreme deficiency symptoms. They may even become completely defoliated, while adjoining limbs with little or no fruit show no symptoms. Again, this characteristic is most pronounced with seedy varieties. It may be aggravated by the tendency of seedy varieties to set fruit more unevenly on the tree than do seedless varieties. The relationships of seediness to magnesium nutrition have been discussed in some detail by both Bahrt (1934) and Fudge (1938).

Alternation in bearing is common in seedy varieties growing under magnesium-deficient conditions. This results from the fact that when trees are bearing a heavy crop, the loss of wood in the summer and fall as a result of defoliation reduces the fruit-bearing wood for the next year. The tree then goes through a season of recovery in which the leaves are green and little or no fruit is produced. The following year the tree may produce a good crop of fruit on the twigs of the previous year and again defoliate and die back. This alternation is a function of individual trees; some may bear fruit and others may not in the same year unless the cycle is set up by freeze damage or drought. Alternation in condition and yield

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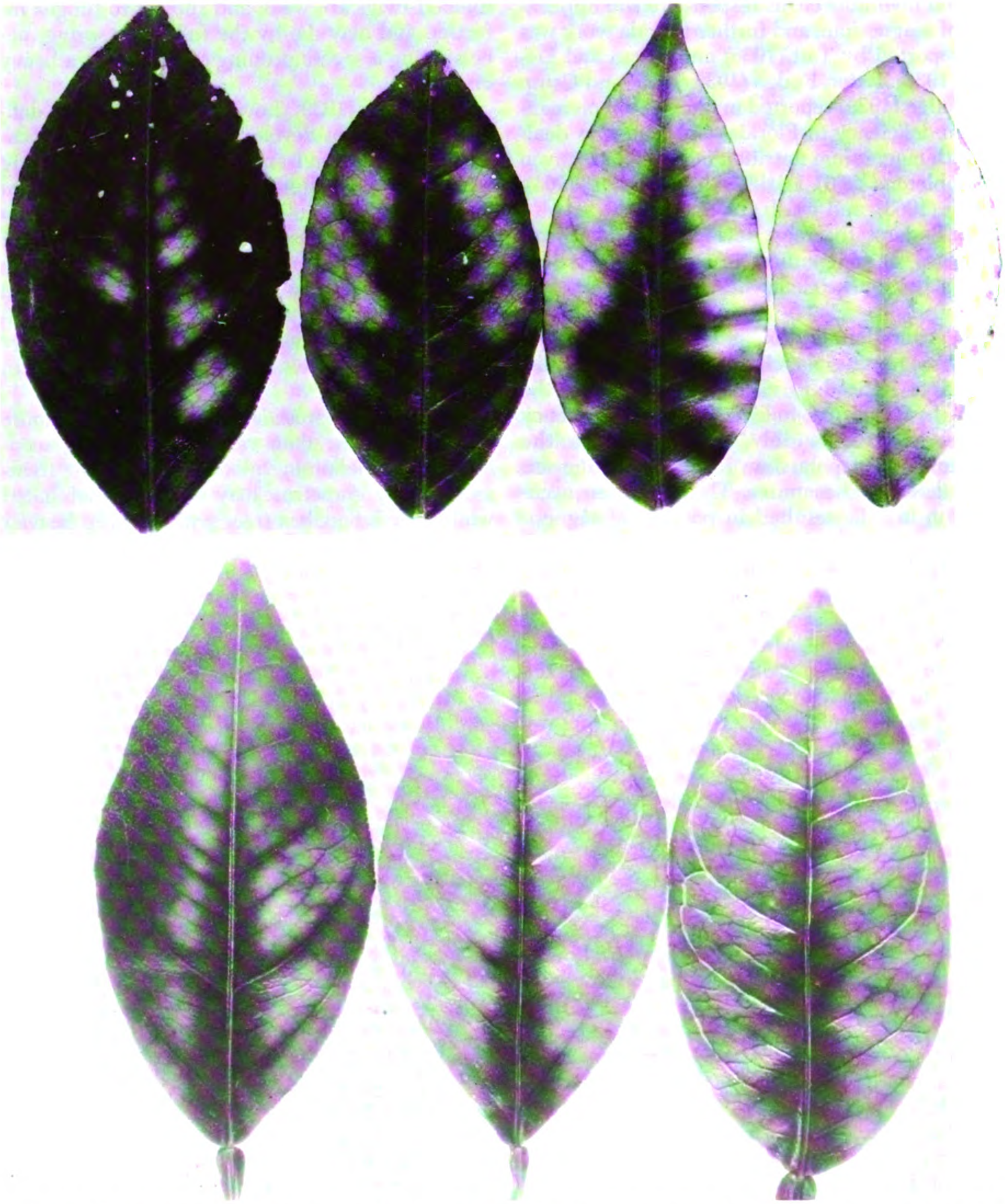

Fig. 3–17. Orange leaves showing various magnesium deficiency patterns. Top, progressive stages of deficiency (left to right). The earliest symptoms are commonly small, interveinal, yellow areas on both sides of the midrib and between the main veins. As the deficiency becomes more acute, only an inverted, V-shaped, green area remains, and later the leaf may completely fade. Bottom, some enlargement and splitting of midrib and veins is commonly found.

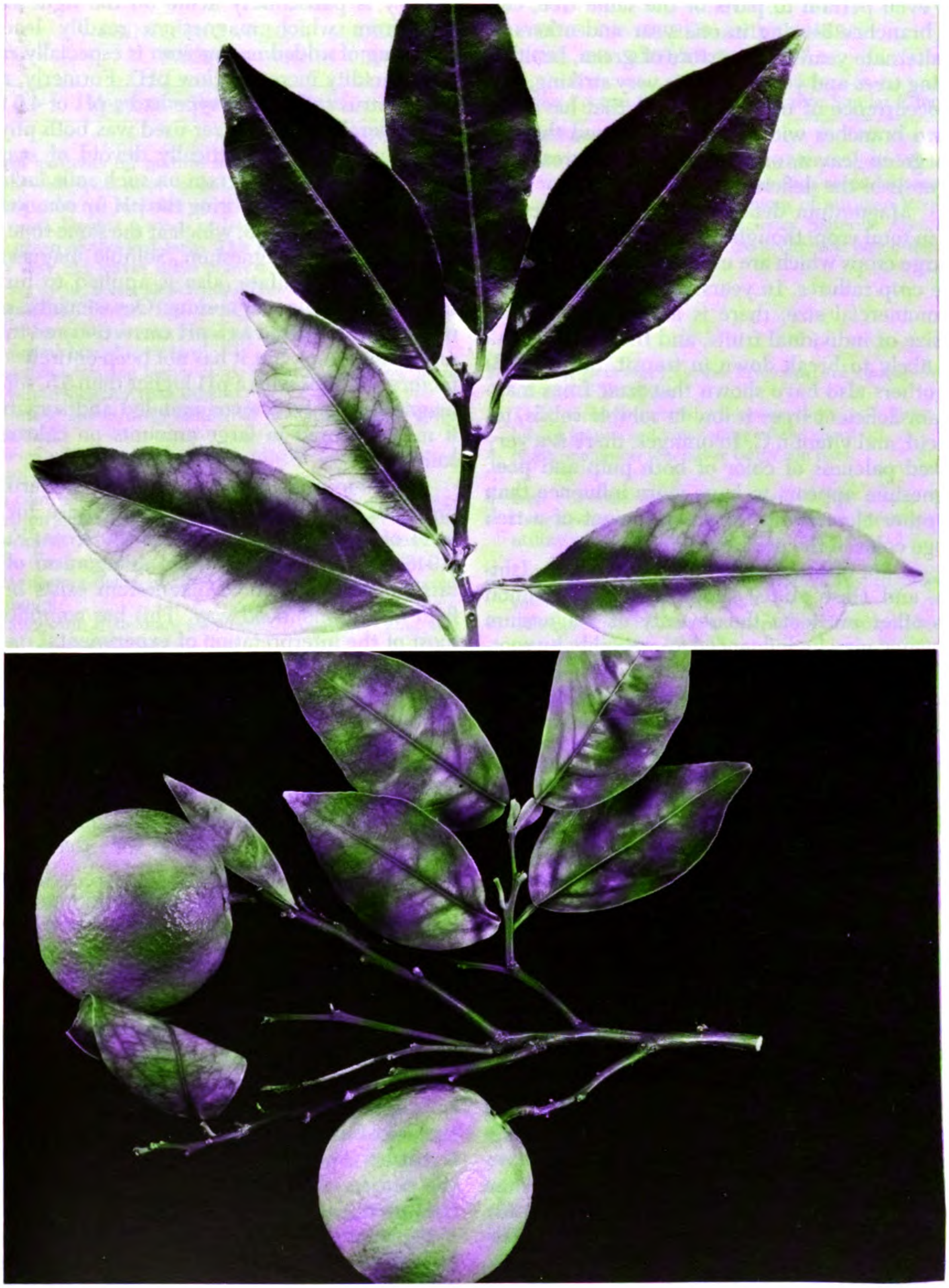

Fig. 3–18. Magnesium deficiency effects on orange foliage. Top, inverted green V-pattern on older leaves (which characteristically appears in the fall of the year on older foliage). Bottom, small fruit, faded leaves (with small, yellowish splotches on either side of midribs), and twig dieback.

may even pertain to parts of the same tree, certain branches bearing in one year and others in the alternate year. This mixture of green, healthy-looking trees and yellow trees is very striking, and the occurrence of occasional trees that have one or two branches with yellow leaves and the rest with green leaves, or vice versa, aids greatly in diagnosis of the deficiency.

Magnesium deficiency results in a reduction in total crop, though in some years there may be large crops which are usually followed by complete crop failures. In years when there is a crop of commercial size, there is also a reduction in the size of individual fruits, and the fruit is weak and likely to break down in transit. Sites (1948) and others also have shown that fruit from magnesium-deficient trees is low in soluble solids, total acid, and vitamin C. In oranges, there is a very marked paleness of color of both pulp and peel. Magnesium appears to have more influence than any other element on the development of a rich orange color in the peel and pulp.

Data of Smith *et al.* (1950) and Sato, Ishihara, and Hase (1963) showed that in common with other nutrients the severity of magnesium deficiency may be influenced appreciably by rootstock.

Magnesium deficiency, like other deficiencies, makes trees more susceptible to cold injury than normal trees (Lawless and Camp, 1940). This applies primarily to trees bearing a crop at the time of the freeze, since on the off-year trees actually may not be in a deficient condition. This susceptibility to cold damage applies to both tree and fruit, although it has not been determined whether damage to the fruit is caused by an actual susceptibility or whether it results from the fact that as a result of defoliation fruits are exposed to the sky and consequently are colder than protected fruit because of radiation losses. This difference in cold resistance is of great importance to growers; on numerous occasions magnesium-deficient trees in Florida have been observed to lose all leaves, twigs, and fruit, while adjoining trees on the same fertility program plus magnesium lose only a few leaves.

Some of the major effects of magnesium deficiency in citrus are summarized in table 3–23.

Cause and Treatment of Magnesium Deficiency.—Magnesium deficiency in citrus in Florida is caused primarily by the low native level of magnesium in the soil and may be aggravated when other bases are added as fertilizers. The deficiency is particularly acute on the light sandy soils from which magnesium readily leaches. Leaching of added magnesium is especially rapid as the acidity increases (low pH). Formerly, most of the citrus soils of this type had a pH of 4.5 to 5, and generally the fertilizer used was both physiologically acid and practically devoid of magnesium. The present program on such soils includes the use of dolomite to bring the pH up somewhere in the range of 5.5 to 6, which at the same time also furnishes some magnesium. Soluble magnesium, usually as the sulfate, also is applied to furnish quickly available magnesium. Occasionally, magnesium oxide is used as a pH corrective and to furnish magnesium, but it has not been entirely satisfactory. On soils with a pH higher than 5.5, soluble magnesium only is recommended and sometimes it must be used in large amounts on calcareous soils.

Some adjustment of magnesium fertilizer practices must be made to compensate for the level of potassium available to the trees. Fudge (1946) showed that the usual antagonism of potassium and calcium to magnesium exists in the field in a pronounced way. This has complicated most of the interpretation of experimental results. In practice, it means that levels for magnesium fertilization can be determined only in relation to potassium levels. At high levels of potassium availability, magnesium must be increased. To a lesser degree, the same situation holds with reference to calcium. To get results, the amounts of magnesium applied on calcareous soils should be greater than those applied on soils that are low in calcium.

When the percentage of magnesium in the exchange complex decreases, with a corresponding increase in exchangeable hydrogen or potassium, magnesium is less readily absorbed by the plant.

The ammonium ion interferes with magnesium absorption, but probably this is not important under most field conditions.

Accumulation of potassium in soils from the use of manures or other potassium-containing fertilizers is prevalent in California. In many cases, the degree of magnesium deficiency is mild, although occasionally severe deficiency occurs. Where potassium accumulates, it often requires rather large and repeated soil applications of magnesium sulfate to bring about correction. Bingham, McColloch, and Aldrich (1956) found that the K:Mg ratio of exchangeable bases in magne-

Table 3–23

PRINCIPAL MAGNESIUM DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Magnesium Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Leaf patterns on mature leaves; these often begin as yellowish spots or areas between main veins and along both sides of midribs; or the margins of leaves fade inward, leaving a more or less well-defined inverted V of green at the leaf base. The main veins and midribs on very old leaves often show enlargement and corkiness, as in boron deficiency. The entire leaf may become bronzed (see figs. 3–17 and 3–18). All variations of these patterns can usually be found on magnesium-deficient branches or trees.	1. Acute stages may be confused with nitrogen deficiency; in fact, the latter seems to produce secondary magnesium deficiency, and applications of nitrogen frequently clear up magnesium deficiency patterns. Also, splitting and corking of veins occur on boron-deficient trees.
2. Branches bearing heavy crops of fruit often show more severe deficiency than others.	2. In iron deficiency, some branches are more severely affected than others.
3. Affected leaves drop somewhat prematurely and twigs die back.	3. Twig dieback occurs whenever there is a premature loss of leaves and weakening of the shoot.
4. Twigs which have died back usually become infected with fungi.	4. Not uncommon whenever twigs die back.
5. In controlled sand cultures, severe and prolonged magnesium deficiency results in a secondary iron chlorosis. Terminal leaves show iron chlorosis and respond to iron spray applications.	5. Iron chlorosis results from many other causes where there is interference with absorption, transport, or utilization of iron in the plant.
6. Magnesium-deficient trees are very susceptible to cold damage.	6. Trees weakened by other nutritional disturbances are susceptible to cold damage.
7. Magnesium deficiency sometimes accentuates zinc and manganese deficiencies.	7. Other nutritional imbalances produce zinc and manganese deficiencies.
Fruiting and Fruit Characteristics	
1. Yields are reduced and alternate bearing is aggravated, especially in seedy varieties of citrus.	1. Most other deficiencies reduce yields, and alternate bearing is a characteristic of some citrus species and varieties.
2. Fruit size is substantially reduced, but there are no special interior effects as with boron and copper deficiencies. Sunburning and wind scarring occur as a result of defoliation.	2. Much the same effect on size is produced when potassium is lacking.
Tissue Analysis Values	
1. Leaves showing magnesium deficiency patterns usually have less than 0.1 per cent magnesium in the dry matter of the leaf. Values less than 0.2 per cent suggest incipient magnesium deficiency.	1. No other condition is known to produce as low values, although magnesium is commonly associated with low nitrogen in leaves.

sium-deficient soils ranged from 0.3 to 0.9, whereas little magnesium deficiency occurred with ratios of 0.1 to 0.3 (e.g., 10 to 3.3 times as much exchangeable magnesium as potassium).

Under irrigation agriculture with waters of low-magnesium content, leaching and loss from crop removal may exceed the amounts added in irrigation water and manures and gradually deplete the magnesium supply of soils (Broadbent and Chapman, 1950; Pratt and Chapman, 1961; Pratt *et al.*, 1959). The addition of fertilizer and soil amendments accelerates magnesium loss. In view of these findings, regular use of some magnesium in the citrus fertilizer program, especially where potassium is included, appears to be a sound practice.

Because of the slowness with which magnesium deficiency is corrected under conditions in which soil potassium buildup has taken place, spray applications of magnesium nitrate have proved beneficial. Embleton and Jones (1959) recommended a spray mixture containing 7.5 to 10 pounds of magnesium nitrate per 100 gallons of water. This mixture produces correction in six months, and meanwhile soil applications can be initiated. Mixtures of calcium nitrate and magnesium sulfate are effective.

In California, nitrogen fertilizers often bring about partial or complete correction of magnesium deficiency. This could be due to the fact that the nitrate added or produced mobilizes calcium and magnesium, which helps to suppress potassium absorption and permits more ready uptake of magnesium.

The effect of nitrogen in ameliorating magnesium deficiency has been noted by many researchers. Embleton (1966) reviewed this subject.

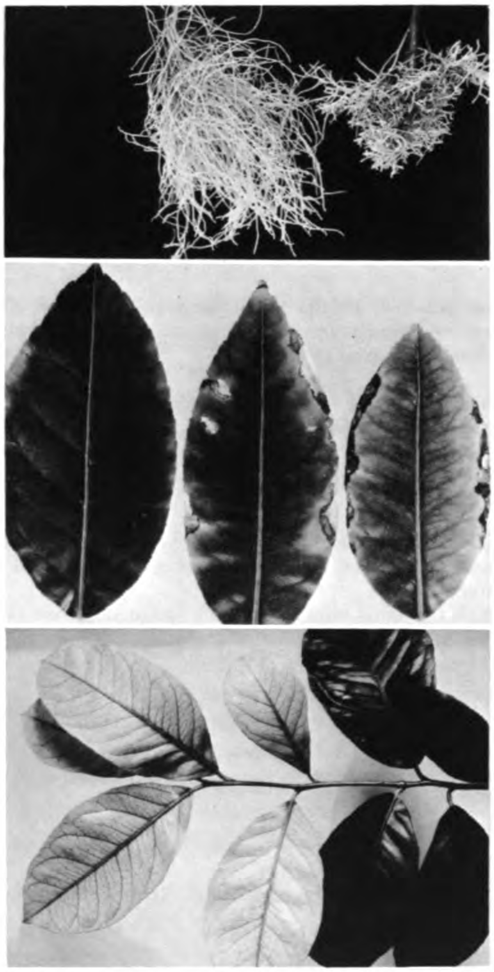

Fig. 3–19. Various effects of excess magnesium on citrus. Top, roots of lemon cuttings grown in nutrient solution containing 30 meq/l of magnesium chloride (right), as compared with same concentration of calcium chloride (left). Center, leaf burn and chlorosis of lemon leaves produced from 100 meq/l of magnesium nitrate added to complete nutrient solution. Bottom, iron chlorosis of new growth of lemon cuttings grown in nutrient solution containing 100 meq/l of magnesium nitrate. Comparable amounts of magnesium chloride and magnesium sulfate also produced iron chlorosis.

Magnesium Excess

The author is not aware of specific cases of magnesium toxicity under field conditions. However, in controlled cultures the author has produced magnesium toxicity. In sand-culture experiments with sweet orange seedlings (incorporating magnetite as a source of iron), Guest (1944) found that additions of basic magnesium carbonate at a rate of 0.1 per cent of the weight of the sand produced severe iron chlorosis and root stunting. Up to 0.5 per cent of calcium carbonate had no effect under comparable conditions. The effect of the magnesium was ascribed to a specific toxic effect of magnesium on the roots.

In subsequent experiments with solution cultures, Chapman (1944, unpublished data) found that 30 meq/l of magnesium chloride added to a complete culture solution severely reduced root growth and produced iron chlorosis of the tops. Comparable amounts of calcium chloride produced no effect (fig. 3–19). In further experiments, 50 to 100 meq/l of magnesium nitrate produced not only iron chlorosis but a marginal leaf burn (fig. 3–19). Comparable amounts of calcium nitrate were without effect. From these limited observations, it is evident that excesses of both solid-phase and soluble magnesium are much more toxic than equivalent amounts of calcium.

Under field conditions, one might look for magnesium toxicity in serpentine-derived soils and also in soils where considerable magnesium carbonate is present. In South Africa, Bathurst (private correspondence) reported a condition called "yellow branch." It occurs in soils of low calcium and high magnesium content.

MANGANESE (Mn)

Manganese Deficiency

As with a number of other trace elements, the wide distribution of manganese in soils and plants was known to early workers. Research by Bertrand (1897*a*, 1897*b*, 1905, 1912) and Mazé (1914), followed by the work of McHargue (1919, 1921, 1923, 1926) and others, showed beyond much doubt that manganese was essential for green plants. Good literature reviews dealing with manganese are given by Mulder and Gerretsen (1952), Scharrer (1955), and Labanauskas (1966).

The first controlled culture manganese work with citrus was by Haas (1932*a*, 1932*b*), who found that lack of this element caused chlorosis of terminal growth. In Florida, field responses to

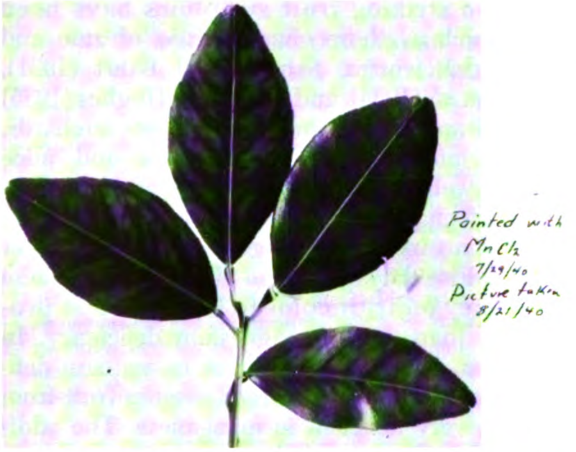

Fig. 3–20. Mild manganese deficiency patterns on an orange shoot, showing rapid disappearance of chlorosis when leaf was painted with a dilute solution (1 mgm manganese per ml of manganese chloride).

manganese were reported by Skinner and Bahrt (1931) and Skinner, Bahrt, and Hughes (1934). Later, Chapman, Liebig, and Parker (1939) produced manganese deficiency both in young orange trees growing outdoors in sand cultures and in lemon cuttings growing in the greenhouse. The leaf patterns closely resembled those noted in Florida by Camp and Reuther (1937), and analyses of affected leaves showed them to have a much lower manganese content than green leaves. Also, the leaves became green when treated with manganese solutions, and additions of manganese to the nutrient medium cleared up the condition. Primarily on the basis of clear identification of leaf patterns of manganese deficiency in lemon and oranges produced under controlled cultures, chlorotic leaf conditions thought to be caused by lack of zinc (Parker, Chapman, and Southwick, 1940) were identified in the field as manganese deficiency. Widespread deficiencies of this element were then found in California citrus orchards and plantings of other fruit trees. After this early work in Florida and California, manganese deficiency was found and identified in a great many citrus-growing regions of the world (Koto and Takeshita, 1957; Carpena, Guillen, and Costa, 1959; Taylor and Burns, 1938).

Symptoms of Manganese Deficiency.—The effects of manganese deficiency on citrus have been well described by Camp, Chapman, and Parker (1949). The following account is taken essentially verbatim from their description.

Both young and old leaves show symptomatic leaf patterns. Young leaves have a fine network of green veins on a lighter green background, but the pattern is not as distinct as in zinc or iron deficiencies and the leaf is greener. As the leaf matures, the pattern resolves itself, in the milder forms of manganese deficiency, into dark green, irregular bands along the midrib and main lateral veins, with lighter green-colored areas between the veins (fig. 3–20). With increasing severity, several gradations in color occur. Similar gradations for lemons are shown in figure 3–21. These range from light green to dull, pale green splotches between the main lateral veins. Although the pattern approaches that of zinc deficiency, it never develops the extreme contrast which characterizes the latter. If the deficiency is still more severe, the leaf assumes a dull-green or yellowish-green color along the midrib and main lateral veins, and the bands of these

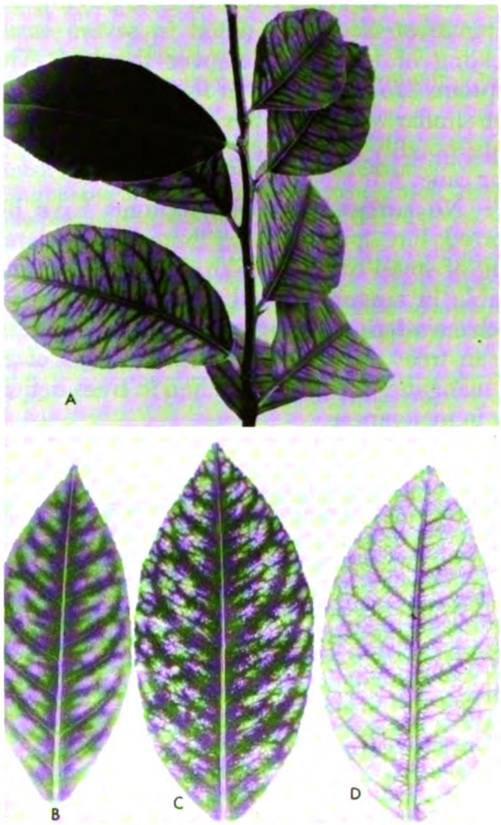

Fig. 3–21. Manganese deficiency patterns on lemon leaves. *A*, curative effect of manganese sulfate spray applied to a single leaf; *B*, leaf pattern commonly seen in the field; *C*, less common pattern; and *D*, leaf pattern characteristic of more acute deficiency.

colors become narrower. At the same time, the interveinal areas become still paler and duller. In extreme cases in California, the interveinal leaf areas of oranges, lemons, and grapefruit develop many whitish spots. Sometimes lemon leaves show a whitish or gray appearance. Parker, Southwick, and Chapman (1940) also observed that in California the leaf symptoms are generally more pronounced on the shaded side of the trees. Such leaves are not noticeably reduced in size or changed in form by manganese deficiency. Severely affected leaves acquire a prematurely senescent (aged) appearance and fall from the tree. Some limbs lose more foliage than others, with the result that the trees are unevenly foliated. When trees are only slightly affected, the young leaves show a mild pattern which disappears after the flush of growth is completed and the leaves mature. This is especially apparent on the spring growth flush.

In addition to the foregoing symptoms, Chapman *et al.* (1939) noted in severe cases a speckling of brown spots over the leaf. These symptoms are illustrated in figure 3–22. A somewhat similar speckling has been noted on other plants, notably sugar beet, oats, barley, corn, and sugar cane.

No particular twig symptoms have been reported, but in acute cases there is considerable dying of small twigs. In Florida, this is more noticeable in tangerines and Temple oranges than in other varieties. Dying of twigs is more noticeable in trees affected with combinations of zinc and manganese deficiencies than in trees deficient only in manganese.

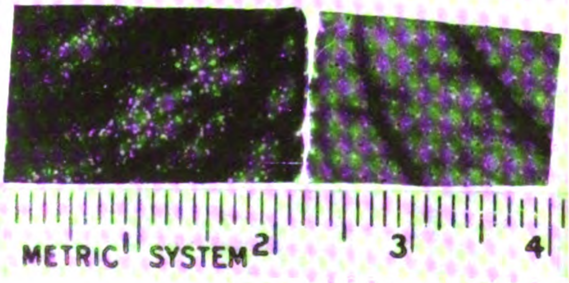

Fig. 3–22. Enlarged sections of upper surfaces of manganese-deficient lemon leaves, showing (left) small, whitish spots sometimes seen, and (right) small, necrotic brown spots which often appear when the deficiency is acute.

No striking fruit symptoms have been noted, such as those characteristic of zinc and copper deficiencies. Skinner and Bahrt (1931), Skinner *et al.* (1934), and Bahrt and Hughes (1935) reported an increase in yield in some orchards, and an intensification in skin color and juice color as well as increased firmness after applications of manganese to deficient trees. Roy (1937) reported an increase in sugar in the juice. Later work in Florida showed the effect of manganese deficiency on fruit color to be much less pronounced than that of magnesium deficiency. In California, fruit color effects of manganese deficiency have not been observed even in fruit from orchards very deficient in manganese. The addition of this element resulted in no improvement of commercial grade or increase in fruit size (Parker, 1945, unpublished data). The yields of citrus trees apparently are not reduced appreciably by very mild forms of manganese deficiency in which the leaf symptoms are transitory. Yield reduction may occur, however, when the leaves are chronically affected. Orchard studies in California by Labanauskas, Jones, and Embleton (1963) and Labanauskas and Puffer (1964) have shown yield increases ranging between 7 and 19 per cent as a result of foliar applications of manganese applied annually. A slight increase in total soluble solids was also noted. A summary of the principal effects of manganese deficiency is presented in table 3–24, and additional illustrations of manganese deficiency effects are shown in figures 3–23 and 3–24.

Causes of Manganese Deficiency.—Manganese deficiency occurs on both acid and alkaline soils; it is probably due to leaching losses and consequent low levels in acid soils and to very low solubility in alkaline soils. On very acid sands, it is commonly associated with deficiencies of zinc, copper, and magnesium, as evidenced by both tree responses and soil analyses. The most severe cases in California however, have not necessarily been associated with marked zinc deficiency, though the two occur together frequently. The artificial acidification of naturally neutral or alkaline soils in California has been observed by Parker and others (unpublished data) to decrease the severity of the deficiency symptoms. Culture studies by Chapman *et al.* (1939) showed that manganese deficiency occurred in a neutral or alkaline medium (pH maintained at 7 or above) even though manganese was regularly supplied.

Manganese in soils occurs as oxides and

Table 3–24

PRINCIPAL MANGANESE DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Manganese Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Leaf mottling, characterized by uneven, lighter-green blotches between main veins; sometimes small light yellow or whitish-colored spots show up along with mottling. The contrast between deep green and lighter green, or yellow is not as marked as with zinc deficiency (see figs. 3–20 through 3–24).	1. Mild zinc deficiency produces similar effects.
2. In more acute cases, especially in lemon leaves, a marked pattern of dark-colored veins against a lighter-colored background is evident.	2. Iron deficiency produces somewhat similar patterns, but the venation is usually more highly delineated and detailed than with manganese. There is no band of green alongside the green vein as with manganese and zinc deficiencies.
3. Both young and mature leaves show patterns; the mature leaf pattern is less apt to be confused with iron and zinc deficiencies than the patterns on young leaves.	3. Zinc and iron deficiencies show patterns on both young and old leaves.
4. In mild stages, leaf sizes are not much reduced, and leaf patterns often disappear as the leaf ages.	
5. Leaves with patterns are more commonly seen on the shady side of the tree.	5. Zinc deficiency patterns are more pronounced on the sunny side of the tree.
6. Older mottled leaves sometimes take on a grayish cast.	6. Severe red spider injury, cold weather, and age will sometimes produce a grayish cast.
7. Where the deficiency is acute, some twig dieback occurs, but it is not as severe as with zinc deficiency.	7. Twig dieback occurs from many other causes.
Fruiting and Fruit Characteristics	
1. Fruit may be somewhat smaller.	1. Small fruit results from many other causes.
2. Fruit may be soft and have a pale rind color when the deficiency is acute, according to Bahrt and Hughes (1935); Roy (1937); Skinner *et al.* (1934).	2. Phosphorus deficiency produces spongy fruit, but usually the orange color is deepened in this case.
Tissue Analysis Values	
1. Leaves showing typical deficiency patterns usually have less than 15 to 20 ppm of manganese in the dry matter of the leaves.	1. Low manganese levels are often associated with acute zinc deficiency, but the condition may be mixed zinc and manganese deficiencies.

hydrated oxides in exchangeable form and in organic combination. Below pH 5.5 it is predominantly in exchangeable and water-soluble forms, but above this pH it occurs at trivalent and tetravalent oxides. It is well known that above pH 6.5 the various manganese oxides are highly insoluble. Manganese solubility (and valence) also fluctuates with the oxygen (aeration) status of soils.

Manganese deficiency of many plants commonly occurs on the following kinds of soils::

1. Thin, peaty soils overlying calcareous subsoils.
2. Calcareous silts and clays.
3. Acid, sandy soils where manganese was low or has disappeared by leaching.
4. Old, black, granular soils where manure and lime have been applied.
5. Phosphate-deficient soils.
6. Sandy soils containing considerable organic matter.

Practices or conditions that commonly produce and aggravate manganese deficiency are liming of soils sufficient to increase the pH to above 6.5, burning of organic soils, irrigation with waters that increase pH and deposit bicarbonates and carbonates, drought conditions, high potassium fertilization, low or deficient calcium, and soil pathogens.

Practices and conditions tending to increase manganese availability are soil acidification, phosphate fertilization, and decreased soil aeration.

Control of Manganese Deficiency.—Soil applications of manganese compounds have not been very successful under neutral or alkaline soil conditions, owing to their rapid conversion to insoluble forms. However, Leonard and Stewart (1959),

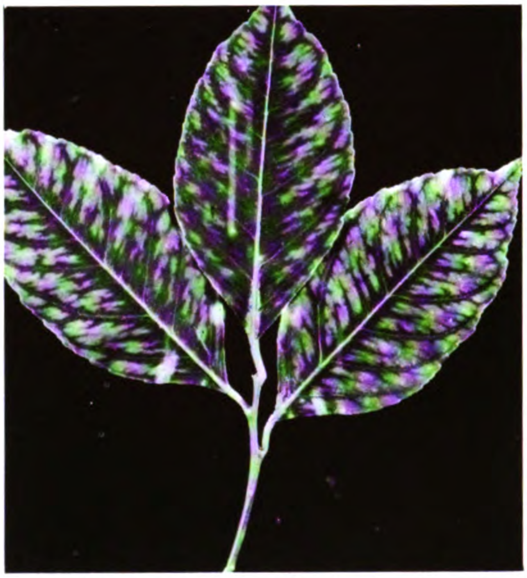

Fig. 3–23. Lemon leaves showing moderately acute manganese deficiency patterns.

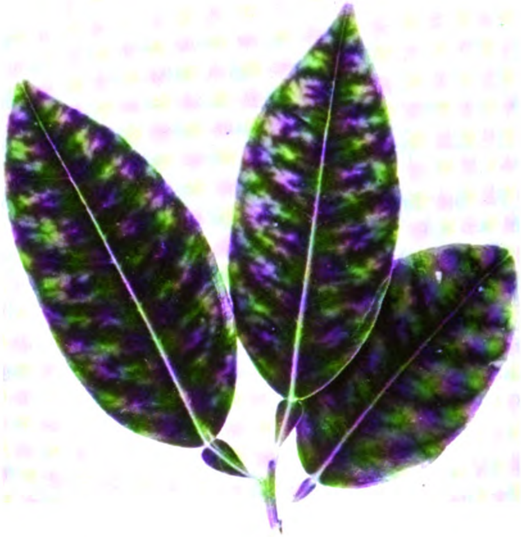

Fig. 3–24. Typical manganese deficiency patterns on recently mature, spring-cycle grapefruit leaves.

in recent work on a calcareous soil, found that applications of a mixture of 5 pounds of manganese sulfate, 5 pounds of calcium chloride, and an additional 10 pounds of wettable sulfur, applied in a series of five to ten piles of about 1 foot square, resulted in high manganese uptake and correction that lasted over eight months; however, the trees became manganese deficient later. Under acid soil conditions, especially on the acid sands of Florida (where the deficiency is caused primarily by low manganese content of the soil), regular additions of manganese compounds to fertilizer mixes have been effective. Chelates of manganese have not been extensively tried as yet on citrus.

Foliar sprays containing 3 to 5 pounds of manganese sulfate and half as much hydrated lime will temporarily correct manganese deficiency, but the treatment must be repeated yearly or more often. To reduce spray residue on leaves, Labanauskas (unpublished data) is now advocating the use of sprays containing manganese sulfate alone at a rate of 1 pound per 100 gallons of water.

Manganese Excess

Although leaf symptoms of excess manganese commonly have been produced on citrus from either excessive soil applications or sprays, or in controlled cultures (Haas, 1932*b*; Smith and Specht, 1953), naturally occurring cases of manganese toxicity on citrus have been rather rare. Perhaps this is because citrus is fairly tolerant to manganese excess.

On the other hand, cases of manganese toxicity, either caused by high soil content of manganese (e.g., pineapples in Hawaii) or soil acidity, have been reported for many other crops by Johnson (1924), Fergus (1954), Sherman and Fujimoto (1947), Parbery (1943), Berger and Gerloff (1948), Schmehl, Peech, and Bradfield (1950), Hewitt (1947), and Löhnis (1951). In recent years manganese toxicity to citrus has occurred in Japan as a result of the heavy use of ammonium sulfate fertilizer.

Some of the leaf symptoms of manganese excess on citrus are shown in figure 3–25. The most characteristic effect is a marginal yellowing of the leaf, with a central green area remaining. Sometimes the veins in the yellowed marginal areas retain their green color. In addition, it is not uncommon to see necrotic spots on various parts of the leaf. Necrotic spotting of leaves and stems has been noted on other plants suffering from manganese excess. (See Hewitt, 1963, for descriptions on other plants.)

Control of manganese excess, where it results from soil acidity, is accomplished by soil liming.

Löhnis (1960) has found that both magnesium and calcium markedly decrease manganese uptake with some crops.

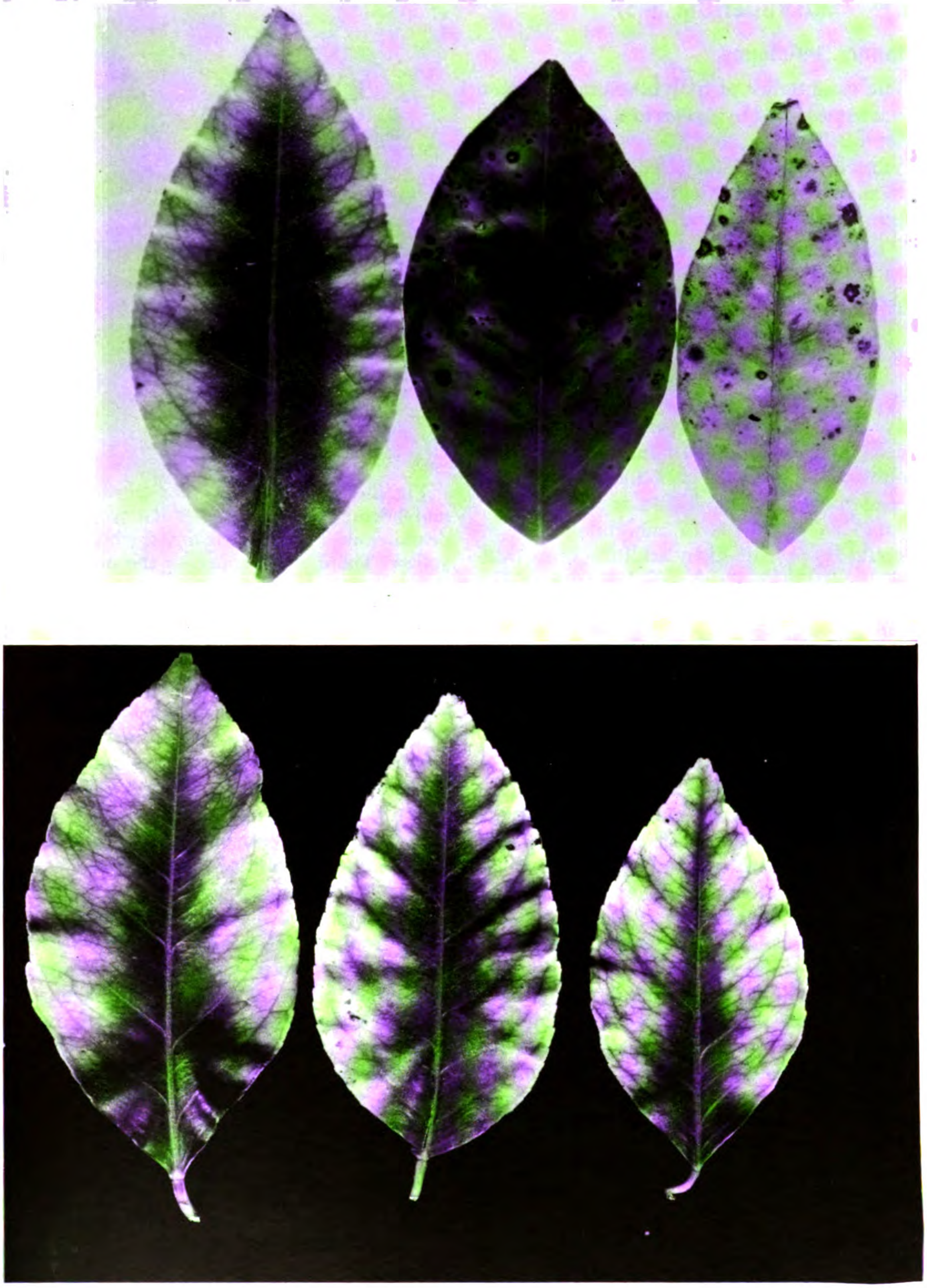

Fig. 3–25. Leaf patterns and burning on orange leaves (top) and lemon leaves (bottom) from excessive manganese absorption. Such patterns are produced from either manganese absorption from sprays or absorption from the soil.

MOLYBDENUM (Mo)[2]

Molybdenum Deficiency

The essentiality of molybdenum for green plants was established by Arnon and Stout (1939*b*) and Piper (1940).

Vanselow and Datta (1949) were the first to produce molybdenum deficiency of citrus under controlled culture conditions. Stewart and Leonard (1952*b*, 1952*c*, 1953*a*, 1953*b*) showed that "yellow spot" of citrus in Florida, a condition long recognized (Floyd, 1908), was due to lack of molybdenum.

The leaf symptoms of molybdenum deficiency, as produced on lemon leaves (and later oranges) under greenhouse conditions by Vanselow and Datta (1949), are different from those seen in the field in Florida, and yet there can be little doubt about the authenticity of these two different effects. Vanselow and Datta found that the growth and foliar effects of molybdenum deficiency could be prevented by addition of as little as 0.0001 to 0.001 ppm of molybdenum to the water cultures. Dilute foliar molybdenum sprays were also effective in producing recovery. The molybdenum content of affected leaves was 0.01 to 0.04 ppm of molybdenum in the dry matter as compared with 0.2 to 0.3 ppm in control cultures showing normal growth. Analyses for other trace elements in these leaves were not enough out of line to account for the leaf burn noted, but it could be that some constituent such as nitrate accumulated sufficiently to produce the symptoms noted by Vanselow and Datta (1949). The foliar effects produced in lemon and orange plants in water cultures are very similar to those produced on tomatoes in nutrient cultures by Arnon and Stout (1939*b*).

In Florida, Stewart and Leonard (1952*c*) found that as little as 1 ounce of sodium molybdate in 100 gallons of a dilute lime-sulfur spray (at 10 gallons per tree, equivalent to 0.1 ounce) would cause the yellow spots to become green within three to four weeks after application. The molybdenum content of "yellow spot" leaves was less than 0.05 ppm in the dry matter.

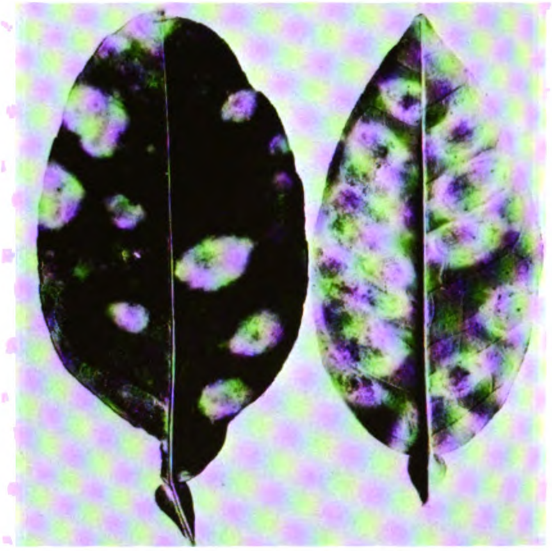

Fig. 3–26. "Yellow spot" of citrus leaves caused by molybdenum deficiency. The first symptom is the appearance of water-soaked areas, which then become yellow. There is gumming on the under surfaces of the leaves (right), and these spots may become quite black in appearance. (Photo courtesy of Ivan Stewart.)

As far as the author is aware, the field occurrence of molybdenum deficiency in citrus has been noted only in Florida and Japan (Otsuka and Takahashi, 1962). However, reports of low molybdenum values in citrus leaves have come from DeVilliers (private correspondence) in South Africa. In California, Lucie-Smith, Vanselow, and Chapman (1950, unpublished data) made leaf analyses for molybdenum on samples from fifty-five commercial citrus orchards (oranges and lemons), representing all of the major California soils in which citrus is grown. The leaves were spring-cycle from fruit-bearing terminals ranging in age from four to twelve months. Values ranged from 0.013 to 0.16 ppm of molybdenum in the dry matter, the majority falling into the range of 0.02 to 0.04 ppm. In no case were symptoms of molybdenum deficiency found, but since seven of the fifty-five orchards showed less than 0.02 ppm of molybdenum, it is probable that sooner or later molybdenum deficiency will be found, not only in California citrus orchards, but in many other areas of the world. In pot-culture trials with a granite-derived California soil, Brusca and Haas

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[2] A bibliography (with abstracts) of molybdenum literature, complete up to March, 1961, and containing over 1,200 citations, has been published by Borys and Childers (1961). A supplemental bibliography (with abstracts) covering molybdenum literature from March, 1961, to June, 1965, prepared by L. G. Albrigo, Richard C. Szafranek and Norman F. Childers is available through Climax Molybdenum Company, 1270 Avenue of the Americas, New York 20, New York.

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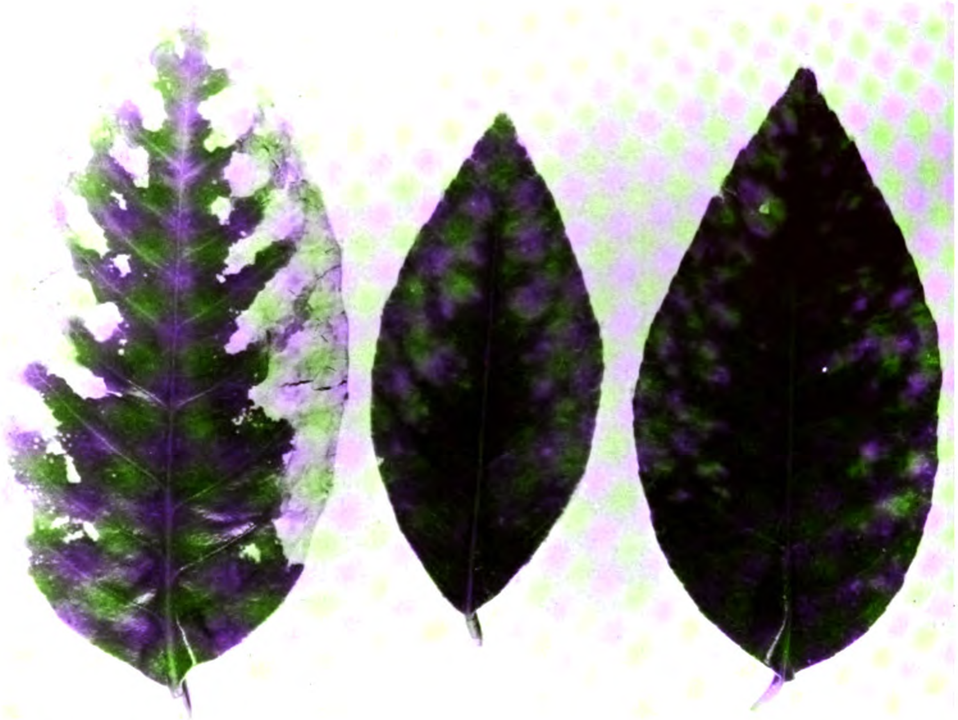

Fig. 3–27. Molybdenum deficiency effects on lemon leaves, as produced in water cultures in the greenhouse. The first symptom is a marginal mottling and spotting (right), followed by marginal and interveinal necrosis. The burned areas curl inward (see fig. 3–28). (Photo courtesy of Vanselow and Datta.)

(1956*a*) got slight growth increases from the addition of small amounts of sodium molybdate.

Effects of Molybdenum Deficiency.—The most characteristic field symptom, as seen in Florida by Stewart and Leonard (1952*c*), is yellow spots on the leaves. They appear first as water-soaked areas in early spring and then develop into larger interveinal yellow spots, with gumming on the lower leaf surfaces in late summer. These gum spots may in time become nearly black (fig. 3–26). In severe cases, complete defoliation of the tree occurs. Trees on grapefruit rootstock appear more susceptible than those on other stocks. Molybdenum in leaves tends to be concentrated in interveinal areas. When it becomes deficient, these areas are the first to be affected. The yellow spots may coalesce as they increase in size.

Fruit is not affected except when the deficiency is severe, and then irregular brown spots with a yellow halo may be found on the rind. The discoloration does not affect the albedo.

The spots on the leaves and fruits are more common on the sunny side of the tree.

According to Stewart and Leonard (1952*c*), the following changes occur in the formation of the yellow spots. First, gum and oil are deposited in the cell cytoplasm, causing the cells to swell and crowd out the air spaces within the leaves. Large deposits of gum are formed in the intercellular spaces. Second, cork cells are formed on the undersides of the affected leaves. Third, there is decomposition of chlorophyll, causing the characteristic yellow spots. Spraying with molybdenum initiates renewed production of chlorophyll and the yellow spots largely disappear.

As produced by Vanselow and Datta (1949) in controlled culture solutions, none of the typical yellow spotting (as seen in the field) occurred. Instead, the effects were similar to those seen in tomato and many other plants, with first an early mottling of the leaf margins, followed shortly by a necrosis and upward curling of the edges of the leaves (figs. 3–27 and 3–28). The necrotic leaves soon fell, and new growth emerged from the axils of the abscised leaves. Only the terminal growth on these cuttings showed the acute stages of mo-

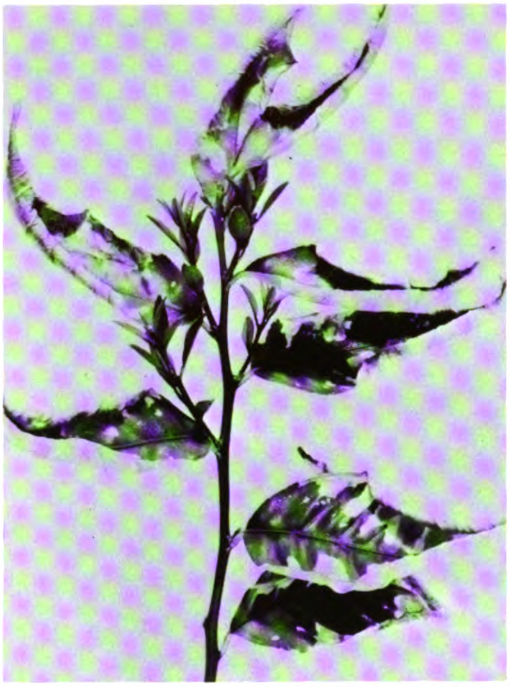

Fig. 3–28. Lemon leaves showing severe marginal and interveinal necrosis produced by acute molybdenum deficiency. New, weak shoots emerge from the leaf axes. (Photo courtesy of Vanselow and Datta.)

lybdenum deficiency; old leaves did not display any symptoms. Total growth of the molybdenum-deficient plants was less than that of controls (fig. 3–29). Vanselow and Datta (1949) found that molybdenum-deficient plants could absorb enough molybdenum from 0.0001 ppm of molybdenum in the nutrient solution to produce normal plants. They also found that the entire plant recovered when the foliage of just one branch of a typically molybdenum-deficient lemon plant was sprayed with 4 mg of molybdenum, as a potassium molybdate ($KMoO_4$) solution. Burned leaves do not recover, of course, but new growth is normal.

Causes of Molybdenum Deficiency.—Molybdenum deficiency is more prevalent on acid soils than on neutral or alkaline soils. Acid sands and acid-residual soils are more apt to be deficient in this element than acid-alluvial soils. Red soils of low pH, derived from basalt and granite, are often lower in molybdenum than those derived from sedimentary rocks. Extensive weathering and leaching reduce the available molybdenum of soils. Molybdenum forms highly insoluble compounds with various secondary clay minerals under acid soil conditions. Liming increases molybdenum availability. Florida soils in which molybdenum deficiency of citrus was found contained from 0.2 to 0.4 ppm of molybdenum (Stewart and Leonard, 1953*b*).

In addition to its direct essentiality for plants, molybdenum is necessary for nitrogen-fixing organisms and other soil organisms, including those concerned with nitrification. Response of legumes to molybdenum is often due to its effect on the nodule bacteria, hence the appearance of nitrogen deficiency symptoms when this element is lacking. It is essential for nitrate reduction within the plant and also performs other functions.

Since phosphorus and sulfur deficiencies commonly occur on acid soils, molybdenum deficiency frequently develops after these have been corrected. In addition to increasing the crop need for molybdenum, there is evidence that sulfate may decrease molybdenum availability by competing for absorption sites on plant roots (Stout *et al.*, 1951). Phosphate, on the other hand, is considered to enhance molybdenum uptake.

Manganese excess has been reported by Mulder (1954) to induce molybdenum deficiency. Manganese solubility increases with increasing soil acidity, whereas molybdenum solubility decreases. Millikan (1948, 1949) has produced evidence of a manganese-molybdenum relation in flax.

Soil-acidifying fertilizers, such as ammonium sulfate, will aggravate molybdenum deficiency by converting molybdenum to less available forms in soils.

Low calcium and high nitrogen may increase the need for molybdenum, according to results obtained with cauliflower by Alexander and Stark (1959).

Control of Molybdenum Deficiency.—Molybdenum deficiency is easily corrected by foliar sprays and, in many soils, simply by liming the soil. Stewart and Leonard (1952*b*, 1953*b*) found that 1 ounce of sodium or ammonium molybdate in 100 gallons of water, applied as a spray at the rate of 10 gallons per tree, corrected the deficiency in three to four weeks. One spraying a year was sufficient. This element, unlike iron and some others, is mobile in plants, and, though it does not appear to move from young to older leaves, it does move readily in the opposite direction.

Soil applications of molybdate salts on citrus in Florida have not proved as effective as sprays, though with most crops soil applications are effective.

In general, rates of 1 to 2 ounces of sodium molybdate per acre are ample, and, in many cases, the molybdate is incorporated in phosphate or other fertilizer mixes to insure more uniform distribution when applied to the soil. The amounts of molybdenum needed are increased where sulfate, iron, and manganese are high or phosphate is low. For these and other reasons, acid soils require higher rates. Frequency of application will depend on the crop, soil, and associated soil conditions. In Australia, for example, it was found that 2 ounces of molybdenum trioxide (MoO_3) per acre, applied in 1946 for subterranean clover, was still providing correction in 1955 (Anderson, 1956).

Molybdenum Excess

Molybdenum is not nearly as toxic as boron, copper, zinc, and other trace elements. There are not much data on molybdenum excess for citrus. P. F. Smith of Florida (private correspondence) has grown orange seedlings with 50 ppm of molybdenum in a culture solution without harmful effects.

Stewart and Leonard (1953*b*) found that sprays containing as much as 1 ounce of sodium molybdate per gallon (0.01 ounce is sufficient for correction) did not cause injury, though the injection of 1 ounce per gallon into the trunks of trees caused many branches to die. Gerloff, Stout, and Jones (1959), working with tomatoes, found that excesses of molybdenum induced iron chlorosis. They suggest that molybdenum and iron form a precipitate of low solubility in root tissues.

For information on other crops, the reader is again referred to the bibliography on molybdenum by Borys and Childers (1961), Albrigo, Szafranek and Childers (1965) and also to reviews by Anderson (1956) and Johnson (1966).

NITROGEN (N)

Nitrogen Deficiency

Nitrogen fertilizer is generally required in greater amounts by citrus than any other fertilizer. Since nitrogen disappears from soil through leaching, volatilization, crop removal, and runoff, sooner or later supplemental nitrogen from one

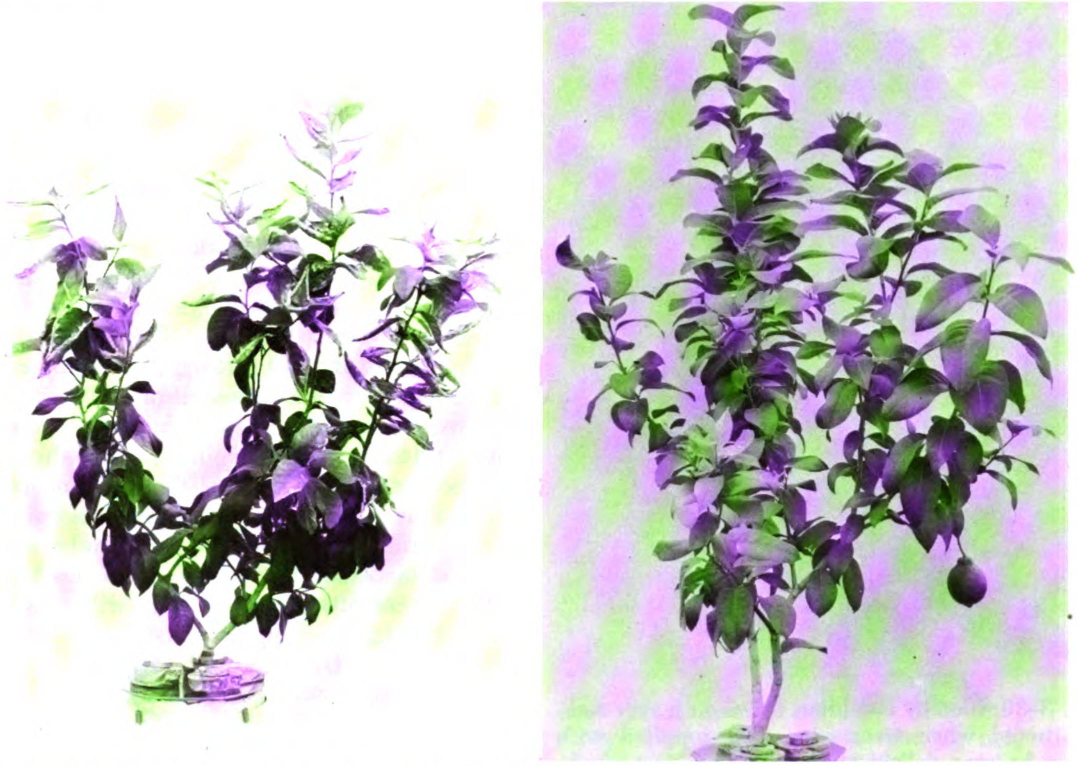

Fig. 3–29. Molybdenum-deficient lemon plant (left), showing reduced size and severe leaf burning and curling of terminal growth, in comparison with normal plant (right). (Photos courtesy of Vanselow and Datta.)

source or another invariably is required for commercial citrus production.

Nitrogen plays a dominant role in citrus nutrition; deficiency, when acute, arrests vegetative growth and greatly reduces fruit production. Excesses, on the other hand, may adversely affect quality, especially with oranges, grapefruit, and mandarins. Also, nitrogen can affect nutrition with respect to phosphorus, potassium, calcium, copper, zinc, and other elements, either directly or through secondary effects of various nitrogen carriers on soil reaction, structure, or nutritional balance.

It is easy to understand, therefore, why so much research has centered around the nitrogen nutrition of citrus. In spite of this, many questions remain unresolved.

Although there are great differences from soil to soil in the time required for native soil supplies to become deficient, the author is aware of no citrus-growing area where the use of some form of nitrogen fertilizer has not ultimately become necessary. Some virgin soils are sufficiently well supplied, however, that no need becomes apparent for the first ten to fifteen years of orchard life. When nitrogen fertilization was discontinued for two to six or more years in heavily fertilized, mature orchards, Jones and Embleton (1959) and Taylor, Pratt, and Goodall (1960) found no impairment of fruit production. Judging by leaf analysis and foliage color, enough residual supply had accumulated to amply supply tree needs for a lengthy period. There are some situations in which irrigation water contains enough nitrogen to meet or reduce nitrogen needs. In low rainfall areas, requirements for nitrogen are usually lower than in areas of leaching rainfall.

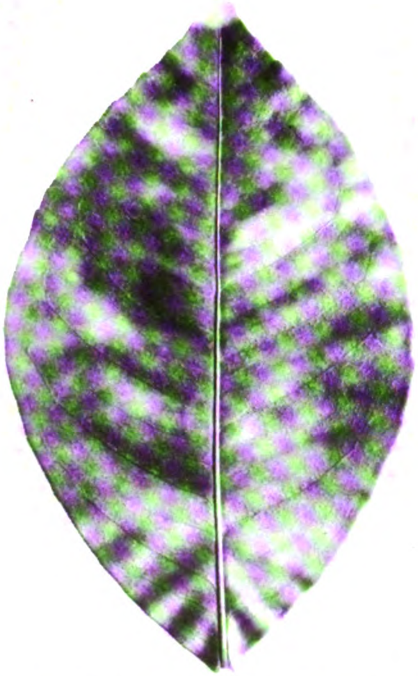

Fig. 3–30. Blotchy condition in lemon leaves commonly noted when trees once well supplied with nitrogen become deficient. This is a transient condition, and such leaves become completely yellow if the deficiency becomes acute or green up if the nitrogen supply improves.

Effects of Nitrogen Deficiency.—Many investigators have described nitrogen deficiency of citrus. Among them are Camp and Fudge (1939); Chapman and Kelley (1943); Bryan (1940); Camp *et al.* (1941, 1949); Chapman, Brown, and Rayner (1945); Smith and Reuther (1954); Malavolta *et al.* (1962); and Uexküll and Kämpfer (1963).

Since nitrogen is a constituent of proteins, chlorophyll, and other essential components of plants, acute lack or shortage of this element brings vegetative growth to a halt and results in a general bronzing and yellowing of foliage, followed by dieback of twigs and decreased and finally almost complete cessation of flowering and fruiting. When trees are well supplied with nitrogen and then gradually become deficient, various transient leaf symptoms appear. In lemons particularly, green leaves may develop yellowish, irregular, blotchy areas before becoming entirely yellow (fig. 3–30). In addition, older leaves may develop vein chlorosis or "yellow vein," as it is sometimes called (fig. 3–31). This condition can also be caused by root rotting, branch injury, calcium deficiency, and other disorders. Sometimes a yellow-vein condition of old leaves will develop when a particularly vigorous new shoot has emerged or much fruit has been borne on the shoot. This symptom is somewhat less conspicuous in oranges and other species than on lemons, but it does occur. If the leaves are not too old, restoration of nitrogen sometimes will cause such leaves to regreen fully or partially. Completely yellow leaves, if not too old, will regreen when nitrogen becomes available; if temperature conditions are favorable, abundant new growth will commence. The author has noted the yellow-vein condition rather widely on oranges and mandarins during the winter in Australia and India. The condition commonly clears up in summer. Leon-

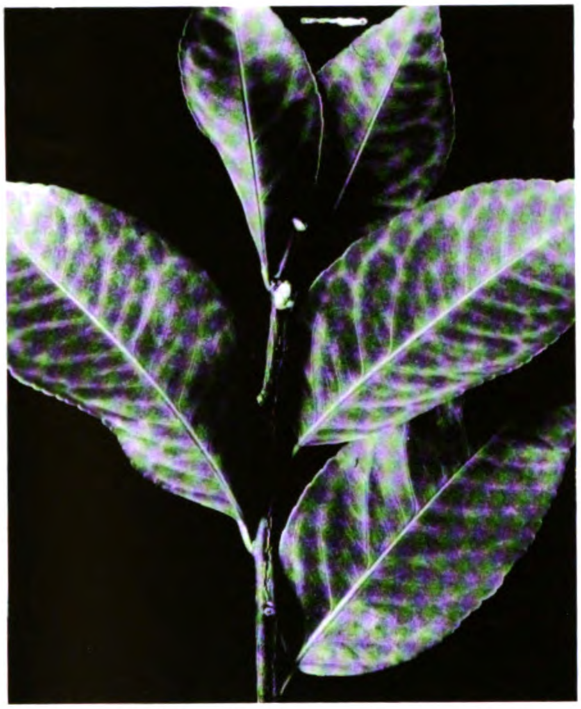

Fig. 3–31. Vein chlorosis (sometimes referred to as "yellow branch" or "yellow vein") is commonly seen on older leaves of lemons when nitrogen is lacking. Heavy crops of fruit or strong new growth are often a cause. This condition is also commonly seen on grapefruit, mandarins, and (to a lesser extent) oranges in winter. It can be largely corrected by nitrogen and often clears up in summer. Root rotting, bark diseases, and injury can also produce this condition.

ard and Stewart (1961) have noted it on red and pink grapefruit in Florida. Analyses of affected leaves showed them to be low in nitrogen and calcium, but normal in phosphorus, magnesium, and boron. Field experiments by Leonard and Stewart (1961) have demonstrated that the condition can be markedly improved by extra nitrogen fertilization, but not by adding calcium chloride or calcium sulfate. Nitrogen fertilization not only decreased the yellow-vein symptom, but increased both the nitrogen and calcium of the leaves. Sand-culture trials have confirmed the aforementioned findings. Although this condition can arise from other causes (e.g., rotted roots, calcium deficiency, and in weak trees afflicted with virus disease), it is apparently often no more than another manifestation of seasonal nitrogen deficiency.

New leaves of trees moderately or acutely deficient in nitrogen are apt to be thin and fragile when young, and the angle between stem and leaf may be narrower than when nitrogen is ample (figs. 3–32 and 3–33).

Trees continuously lacking in nitrogen are stunted, unsymmetrical in shape and twig growth, short, and irregular. Such trees seldom die and remain in this condition with some seasonal, weak new growth appearing from time to time. Fruit production is greatly decreased, but quality is not seriously impaired. The fruit rind is smooth, may be somewhat pale in color, and fruit size may be decreased. Juiciness may actually increase, and total solids are not much affected.

Nitrogen deficiency at certain stages resembles phosphorus or sulfur deficiency, but an analysis of mature leaves will establish a clear-cut differential diagnosis. If the trouble is lack of sulfur or phosphorus, nitrogen content will be equal

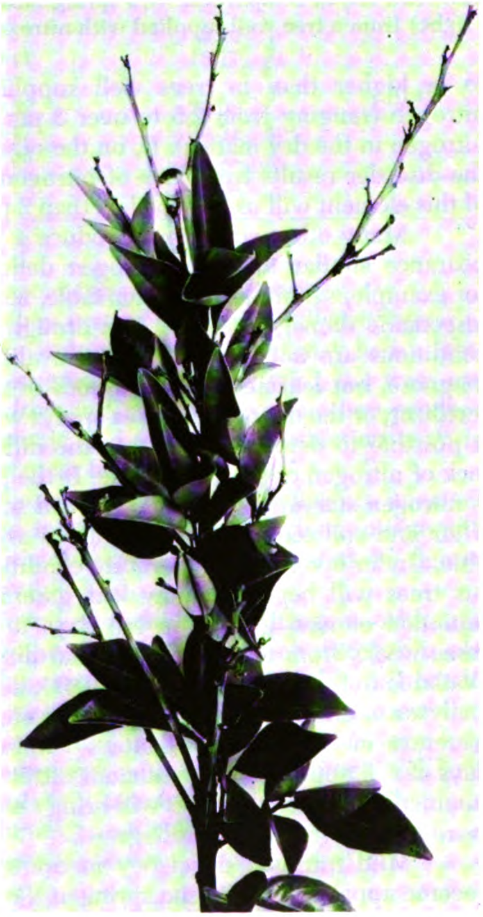

Fig. 3–32. Orange shoot showing twig dieback, small leaves, and narrow angle between leaf axil and stem from a tree acutely deficient in nitrogen. Lower leaves are quite green; terminal leaves are bronzed in appearance.

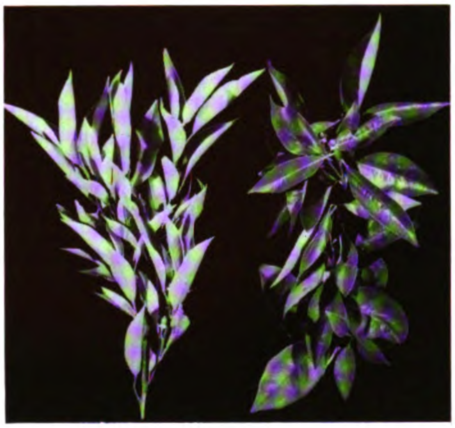

Fig. 3–33. Spring flush of growth (left) on nitrogen-deficient orange tree, showing yellow color, pointed, narrow, and semirolled leaves, and lack of new fruit set, in comparison with spring flush growth (right) from a tree well supplied with nitrogen.

to or higher than in trees well supplied with nitrogen (ranging from 2.5 to over 3 per cent of nitrogen in the dry matter). If, on the other hand, the disorder results from lack of nitrogen, values of this element will usually be less than 2 per cent.

Many other conditions produce a tree appearance similar to acute nitrogen deficiency—for example, salinity, high water table, insect and insecticide damage, neglect, and drought. These conditions are not always easy to evaluate and diagnose, but leaf analyses, additions of nitrogen fertilizer, or the use of foliar urea sprays will make it possible to determine whether the difficulty is lack of nitrogen or something else. If the problem is nitrogen starvation, new growth will soon start if air and soil temperature conditions are favorable. Under favorable temperature conditions, citrus trees will begin to show leaf greening and initiation of new growth in ten days to two or three weeks from the time additional nitrogen is available to the roots. Hilgeman (1941), using leaf analyses to check nitrogen uptake, got significant increases in leaf nitrogen content within fifteen days of soil applications of calcium nitrate in late summer. Urea foliage sprays will bring about even more rapid regreening of foliage.

Mild nitrogen deficiency in oranges may become apparent only in the spring at the time of blossom and new growth (fig. 3–34) or in the late summer and fall when the blossoms and fruit are drawing heavily on nitrogen supply.

For spring blossom and new foliage, nitrogen stored in the older leaves, bark, and wood is heavily drawn upon, and, if insufficient, smaller-than-usual leaves are produced and the new foliage is yellowish and weak. Cameron and Appleman (1934), Cameron *et al.* (1952), and Smith and Reuther (1950*b*) reported on this problem. Data on the distribution of nitrogen in a ten-year-old Valencia tree, as reported by Cameron and Appleman (1934), are shown in table 3–25. It is of interest that the leaves alone contained 41 per cent of the tree's total nitrogen, and the rest of the top, 28.1 per cent. Roots accounted for 10.4 per cent, and the fruit for 20.5 per cent. Wallace, Cameron, and Mueller (1951) showed that fruit, flower, and leaf production required large amounts of nitrogen, calcium, and potassium.

The early stages or trends toward nitrogen deficiency in oranges can usually be diagnosed by the analysis of four- to seven-month-old spring-flush leaves. Orange leaves from fruiting terminals should contain more than 2.30 per cent of total nitrogen in the dry matter, and leaves of comparable age from nonfruiting terminals should run 2.50 per cent of nitrogen or higher.

Not enough data are available for grapefruit, lemons, and mandarins to indicate critical

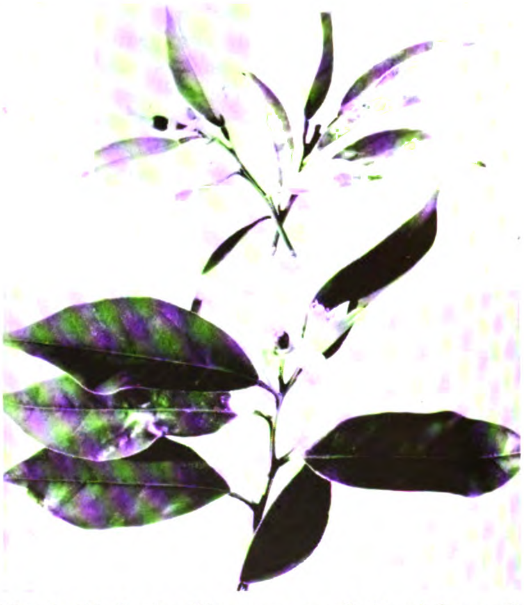

Fig. 3–34. Spring-blossom growth (top) from nitrogen-deficient tree, showing weak, yellowish, small, narrow, and pointed leaves, as compared with growth (bottom) from tree amply supplied with nitrogen. A heavy draft on reserve nitrogen and other constituents is made during the spring blossom and growth cycle, and lack of nitrogen shows up especially at this time. There are also less blossoms and fruit set.

Table 3–25

DISTRIBUTION OF NITROGEN AND DRY MATTER IN A TEN-YEAR-OLD VALENCIA TREE DUG ON JANUARY 25, 1929

Fraction of Tree	Total Dry Weight of Fraction (Grams)	Per Cent of Total Tree In Fraction	Per Cent Nitrogen In Fraction (Dry Weight Basis)	Total Nitrogen (Grams)	Per Cent of Total Nitrogen of Tree In Fraction
Leaves	15,518	19.37	1.94	301.36	41.00
Fruit	12,663	15.80	1.19	150.36	20.47
Terminal growth:					
Vegetative	1,433	1.79	1.28	18.34	28.08
Fruit wood	299	0.37	1.09	3.25	
Branches:					
Bark	4,791	5.99	1.22	58.65	
Wood	25,328	31.62	0.37	94.08	
Trunk:					
Bark	344	0.43	1.19	4.09	
Wood	7,029	8.78	0.39	27.69	
Main root:					
Bark	182	0.22	1.57	2.86	10.42
Wood	4,424	5.52	0.41	18.17	
Roots (0.5–6.0 cm diam.):					
Bark	1,364	1.70	1.62	22.09	
Wood	6,444	8.05	0.47	30.31	
Roots (less than 0.5 cm)	289	0.36	1.08	3.12	
Total	80,108			734.37	

Source: Cameron and Appleman (1934).

nitrogen levels, nor is it known at just what stage of nitrogen deficiency fruit set and final yield begin to be impaired. There are some indications that mild nitrogen deficiency (a stage at which there may be just a little yellow or bronze cast to the tree as a whole, in contrast to the deep green of adequate nitrogen) does not materially impair either fruit set or yield (Chapman, Joseph, and Rayner, 1955, unpublished data on oranges). Smith and Rasmussen (1961) in nitrogen rate trials with grapefruit found that trees which showed seasonal nitrogen deficiency in spring and in the fall bore just as well as those receiving ample and high nitrogen rates. However, more information on various varieties under a range of soil and climatic conditions is needed to throw further light on this aspect.

There is some evidence that high nitrogen can offset the ill effects of high sulfate and boron (Foote and McElhiney, 1937; Haas and Thomas, 1928). A recent paper by Jones *et al.* (1963) suggests that under either high sulfate or boron or both of these conditions, higher leaf levels of nitrogen should be maintained. Smith and Rasmussen (1961) reported that toxic effects of lead arsenate sprays on grapefruit were offset or diminished on trees receiving plenty of nitrogen.

When orange trees are mildly nitrogen-deficient, the only noticeable effect may be a slightly reduced growth rate. Otherwise, the trees appear healthy and green. However, they produce less blossoms and fruit and production may in some instances be impaired.

A summary of nitrogen deficiency effects is presented in table 3–26, and various foliage and growth characteristics are illustrated in figures 3–30 through 3–36.

Forms of Assimilable Nitrogen.—Although nitrate is the chief form in which nitrogen is absorbed by citrus under normal arable soil conditions, there is abundant evidence from controlled culture experiments that the ammonia form can be used. In work with orange seedlings and lemon cuttings in water and sand cultures, Chapman (1951) summarized several years of research on this subject as follows:

> (1) At concentrations of 5 meq/1 of ammonium, growth was as good in most cases as with the same concentration of nitrate. Equal mixtures of nitrate and ammonium at the same total concentration gave comparable results. At higher concentrations of ammonium as the sole source of nitrogen, various troubles appeared; in some

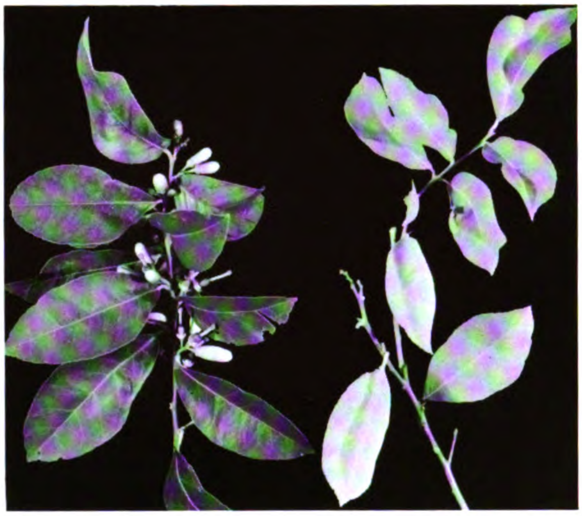

Fig. 3–35. Nitrogen-deficient lemon shoot (right) showing extreme yellowing and lack of blossoms and fruit setting, as contrasted with that seen in the spring of the year on a comparably aged shoot (left) from a tree receiving ample nitrogen.

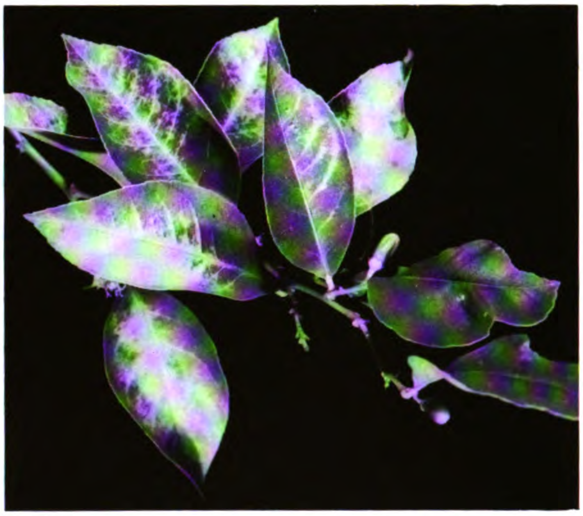

Fig. 3–36. Lemon shoot showing patterns produced when trees formerly acutely deficient in nitrogen and completely yellow were given nitrogen. Leaves may become completely or partially green again, as seen in the shoot.

experiments, manganese deficiency symptoms were produced; in nearly all instances, roots showed signs of injury.

(2) With plants which had been previously growing in cultures receiving nitrate, a sudden change to ammonium halted growth temporarily; sometimes iron chlorosis developed. This, however, disappeared when the plants became adjusted to the change.

(3) Severe pruning of healthy plants growing in ammonium cultures often was followed by root rotting, whereas in nitrate cultures maintained at the same concentrations, no root rotting occurred.

Wander and Sites (1956) found that rough lemon seedlings could absorb and use the ammonium ion, and Wallace and Mueller (1957) likewise found that the ammonium ion could be used by rough lemon cuttings. Van der Merwe (1952–53) reported on extensive field and controlled sand-culture experiments dealing with nitrate and ammonium absorption by citrus, noting that where the latter ion predominated, higher leaf levels of nitrogen were found, potassium absorption was decreased, and the acidity of the nutrient medium was increased. Also, the ammonia form seemed to adversely affect fruit quality. In sand cultures, leaf symptoms of zinc, manganese, and magnesium deficiencies developed if the ammonium ion predominated.

Smith (1957) grew Pineapple sweet orange seedlings in aerated water cultures supplied respectively with nitrate, nitrate-ammonium, and all-ammonium nitrogen sources. At pH 6, growth was as good in the all-ammonium cultures as with all nitrate. At pH 4, growth in the ammonium cultures was somewhat less than with nitrate.

Seasonal Absorption of Nitrogen.—Chapman and Parker (1942), working with young bearing orange trees growing outdoors in water cultures, measured weekly nitrogen absorption over a continuous period of forty months. They showed that absorption rate was largely determined by solution temperatures. Data obtained are shown graphically in figure 3–37. Roy and Gardner (1946), working in Florida, obtained similar results. Bitcover and Wander (1950), using gravel cultures, showed that under certain conditions an appreciable amount of nitrogen can be accumulated very rapidly (within minutes) by citrus roots. While absorption is much less in winter months when soil temperatures are lower, there is some nitrate absorption at all times.

Causes of Nitrogen Deficiency.—The nitrogen cycle in soils has been so widely described that there is no need to repeat it here. As has been stated, leaching and volatilization losses plus substantial crop uptake (heavy crops of citrus fruit, e.g., 40,000 pounds, will remove between 45 and 55 pounds of nitrogen per acre per year) are the basic reasons why supplemental nitrogen is sooner or later needed for citrus. Also, the organic nitrogen in most soils of the subtropical and

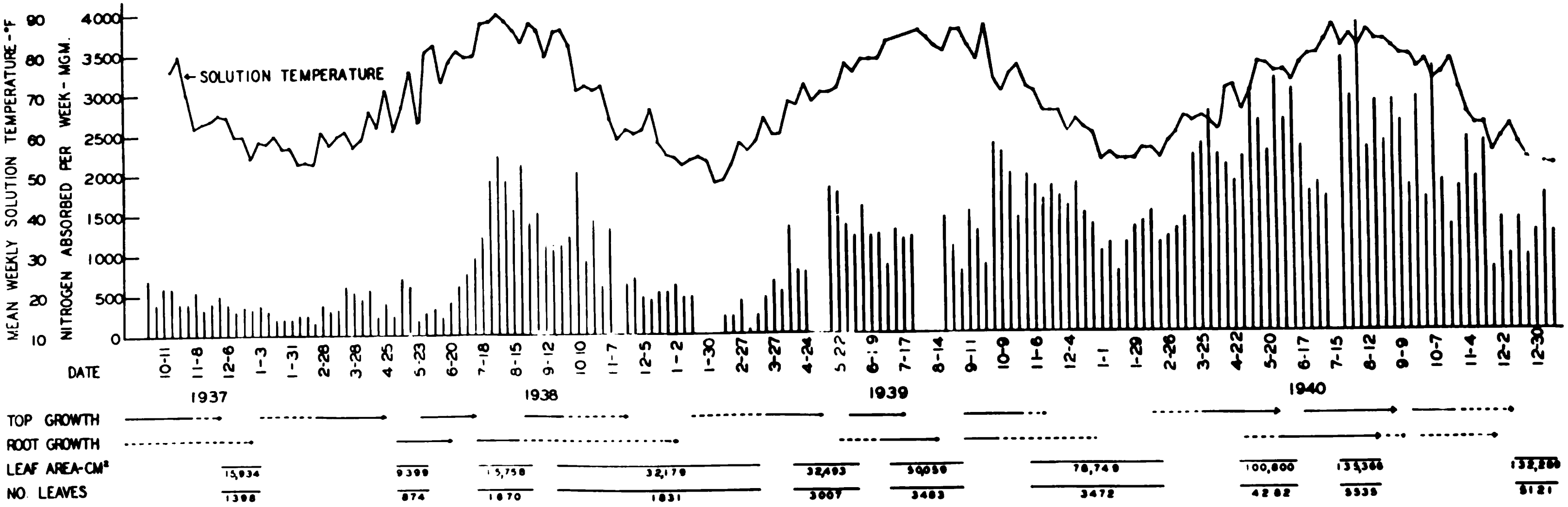

Fig. 3–37. Graph showing weekly nitrate absorption by a young bearing orange tree growing in an outdoor water culture over a 40-month period in relation to solution temperature. The highest rate of absorption was from May through October, and the lowest rate was from the middle of December to the middle of February. Increases in absorption rate were noted when new root flushes appeared, but in the over-all picture the absorption rate was clearly tied to seasonal solution (and air) temperatures. However, there was some absorption all year, even though root growth completely stopped during the winter.

tropical zones where citrus is grown reaches equilibrium levels below those found in colder areas. This is another reason for the universal need of supplemental nitrogen in the citrus-growing areas of the world.

There are other factors which influence nitrogen need and availability. Bulky organic materials of high carbon-nitrogen ratio cause a temporary tie-up of available soil nitrogen by soil organisms. High sodium, chloride, sulfate, and boron in soils require heavier nitrogen usage. The beneficial effects of nitrogen may be indirect, since most nitrogen added to soil, irrespective of form, increases calcium in the soil solution. Calcium, in turn, reduces sodium and boron absorption by roots and tends to limit the solubility of sulfate.

Under waterlogged (anaerobic) conditions, nitrogen is lost by denitrification. Additions of elemental sulfur have been shown by Martin and Ervin (1953) to cause biological reduction of fixed nitrogen compounds.

There is evidence that high phosphorus increases nitrogen need. On the other hand, high potassium may decrease nitrogen need (Chapman and Liebig, 1940).

From this brief account, it is not difficult to understand why the nitrogen requirements of citrus vary enormously from soil to soil and why the old rule-of-thumb recommendations about annual nitrogen fertilizer needs are now being abandoned in favor of the use of leaf and soil analyses for guiding fertilizer practices.

Control of Nitrogen Deficiency.—This topic is treated more extensively in a later chapter, and no attempt will be made to discuss it here, except to point out a few guiding principles. Questions concerning amounts, timing, form, and manner of application continue to be of interest and importance.

Many investigators have shown that with some citrus (especially grapefruit, oranges, and mandarins) excessive nitrogen can impair fruit quality, especially during the period of fruit maturation and under climatic conditions favorable for vegetative growth. However, because of the heavy draft on nitrogen during the spring bloom and growth flush, it is generally held that nitrogen supplies must be adequate then and that excesses during this period are not apt to be harmful. Hence, there is emphasis on early spring fertilization and the use of foliar urea sprays if foliar appearance and/or leaf analyses indicate a nitrogen need at that time. While more data are needed for different citrus varieties, it appears that five- to ten-month-old spring-cycle leaves from fruit-bearing terminals of oranges should contain better than 2.3 to 2.4 per cent of nitrogen in the dry matter. Similar cycle leaves from nonfruiting terminals should show at least 2.4 to 2.6 per cent of nitrogen. The use of nitrogen in general should be decreased later in the season, although this will depend on species, variety, and climatic factors.

While much emphasis was formerly placed on the annual requirements of nitrogen per tree, it is now clear that because of soil, variety, age, yield, and climatic variables such recommendations are almost meaningless. About all that can be said is that there are very few situations where less than 1 pound of nitrogen per tree per year for mature trees will suffice. Conversely, there are few situations where more than 3 pounds of nitrogen are needed. The form, time, and manner of application appears to be largely conditioned by prevailing soil, tree, and climatic circumstances. If a quick buildup of nitrogen in the foliage is needed in the early spring at or following bloom, urea foliar sprays are useful (Impey and Jones, 1960*a*, 1960*b*). If soils are acid (e.g., pH 5.0 or lower), ammonium sulfate and other soil-acidifying fertilizers should be avoided and calcium, sodium, or potassium forms employed. If there is already a sodium problem, sodium nitrate should be avoided. If there is a salinity problem, urea or ammonium nitrate should be used; if more calcium is needed, calcium nitrate can be employed; if soil structure needs improving, organic manures and (under some conditions) calcium nitrate can be employed. If excessive leaching is a problem, slowly available organic forms should be used. Resin- or plastic-coated fertilizer offers interesting possibilities.

The discussion above is sufficient to illustrate that control methods must be tailored to the soil, plant species, plant age, and climatic situations. No universally applicable system can be established.

Jones and Parker (1949*b*), Jones and Steinacker (1953), Jones, Embleton, and Goodall (1955), Haas (1949*a*), Kuykendall and Wallace (1953), and others have investigated the absorption and use of urea as a foliar spray and demonstrated that this method of supplying nitrogen is practical. It is especially useful when a quick buildup in the tree is needed. Several sprayings a year may be required if this is the only method used.

Table 3–26

PRINCIPAL NITROGEN DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Nitrogen Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Dull green, yellowish, and smaller leaves. Leaves are generally uniform in color.	1. Sulfur and phosphorus deficiencies produce somewhat similar effects.
2. Decreased growth or complete cessation of growth when deficiency is severe.	2. Other deficiencies and salt excesses decrease growth.
3. Dieback of twigs and gradual defoliation, resulting in thin, brushy appearance of tops.	3. Other deficiencies and salt excesses cause dieback and defoliation.
4. Where trees were formerly well supplied and the foliage was green, decreased nitrogen supply may lead to vein clearing in older leaves and branches (especially prominent on lemons, but seen at times on oranges, grapefruit, and mandarins). Also, yellow blotches in formerly green leaves may develop (see figs. 3–30 and 3–31).	4. Vein clearing (yellow vein, vein chlorosis) can be caused by root rotting, bark injury or disease, girdling, calcium deficiency, and other factors.
5. If not too old and senescent, yellow nitrogen-deficient leaves green up completely when nitrogen is provided either by urea sprays or to the roots.	5. None known.
6. Bloom is decreased markedly when the deficiency is acute.	6. Potassium and phosphorus lack may produce sparse bloom.
7. Effects of deficiency are more acute on fruit-bearing branches.	7. Magnesium deficiency produces a similar effect.
Fruiting and Fruit Characteristics	
1. Fruit production decreases.	1. Many other factors can decrease yields, especially lack of phosphorus, sulfur, magnesium, and boron.
2. Fruit tends to be smooth, sometimes, but not always, smaller, and of somewhat lower acid content; quality in general is not markedly affected.	
Tissue Analysis Values	
1. Yellow leaves may show values ranging from 1.25 to 1.75 per cent total nitrogen in dry matter. Dull green to green leaves may show from 1.75 to 2.1 per cent of total nitrogen.	

Nitrogen Excess

In addition to the effects of excess nitrogen on growth, fruit yields, and quality, excessive nitrogen in some cases directly or indirectly influences the availability of copper, zinc, manganese, molybdenum, phosphorus, and other elements. In many cases, the effect is due not to nitrogen excesses per se but to effects on soil reaction, or to the forms, manner, timing, and quantities applied. Also, in some soils high rates of fertilization can cause soil structure deterioration and a whole chain of consequential changes.

Ford, Reuther, and Smith (1957) found marked reduction of feeder roots in Valencias on Florida soils receiving high nitrogen rates.

Under special circumstances there may be injury from impurities carried by various nitrogen salts, such as biuret from urea and potassium perchlorate in Chilean sodium nitrate. Nitrite may be formed under some conditions, and various side effects from ammonium ion absorption may occur.

With regard to what might be termed "direct" effects, nitrogen promotes vegetative growth, delays fruit maturity somewhat, and, under some conditions (particularly where climate is favorable for vegetative growth), stimulates tender late-fall growth flushes which are vulnerable to winter frosts.

Also, with Valencia orange fruit, excess nitrogen sometimes promotes a certain amount of regreening (Jones and Embleton, 1959). Regreening decreases sales appeal and, if the fruit is sweated by packinghouse treatments, may lower keeping and shipping qualities. Under some conditions, excess nitrogen will produce fruits with somewhat coarser rinds, thicker skins, less juice, and poorer keeping and shipping qualities.

However, these effects are not universal, and some of them may result from an unbalancing effect on tree nutrition. These nutritional imbalances can be corrected by appropriate treatments. Rootstock also can make a big difference. Excess nitrogen is often worse on oranges budded to rough lemon than on sweet orange (Bouma and McIntyre, 1963).

There are reports from many places of slight to moderate adverse effects of excess nitrogen on quality and yield (Jones, Embleton, and Steinacker, 1957; Wallace *et al.*, 1952; Jones and Embleton, 1959; Oppenheimer and Heymann-Herschberg, 1954; Singh and Agrawal, 1959, 1960; Bain, 1949; Jones, Van Horn, and Finch, 1945; Bouma, 1959; and Sites, Wander, and Deszyck, 1962). Most of the aforementioned investigations are not complete enough to determine the degree to which the adverse effects reported might have been offset by the use of a different rootstock or altered nutrition (e.g., more phosphorus), and especially the ameliorating or aggravating effects of climatic variables. Often the experimental comparison is with fruit from plots where nitrogen is deficient. As is well known, fruit produced on low or nitrogen-deficient trees is generally smooth and of good size and quality, but yields are low. To assure good yields, there must be adequate nitrogen. Under these conditions, sizes may sometimes be smaller (largely owing to increased numbers of fruit set), and fruits may be a little coarser in rind texture, have slightly thicker peel, and have somewhat lower juice percentage. However, these effects are sometimes small and of little commercial consequence. Hence, experimental results with nitrogen variables have to be evaluated in terms of the conditions under which they were obtained, and what might have been the result under other conditions of usage.

There are many conflicting data in the literature relating nitrogen tests to yield, fruit sizes, and quality. A brief review of data is presented here to document some of the preceding statements.

Samuels (1931), in a study of soil nitrate as related to navel and Valencia orange fruit sizes in California, found that in many orchards fruit growth and sizes were greater when soil nitrate was high than when it was low. This may have resulted from the greater use of manure on the high nitrate orchards and hence more potassium rather than nitrate per se. In any case, the data are of interest even though not suspectible to critical analysis.

With respect to yield, increased nitrogen, up to a point, commonly results in increased yields. There are some cases on record where yields have been materially decreased by high or excessive nitrogen usage. Where yields have been decreased, it has often been due to adverse secondary effects on soil phosphorus availability or other causes.

After twelve years of a field trial with navel oranges on sweet orange stock involving nitrogen at rates of 1, 2, and 3 pounds of nitrogen per tree per year, Parker and Batchelor (1942) obtained highest yields at the 3-pound rate. One half of the nitrogen in each treatment came from manure. Winter cover crops were also grown and turned under. It seems likely that the results obtained largely reflected a direct nitrogen effect and not, as has been suggested (Jones *et al.*, 1961), that the increased yields were due to improved soil permeability resulting from the greater organic matter additions from the manure and from the increased growth of winter cover crops. No yield responses in this experiment were obtained from phosphate or potash, and zinc deficiency was controlled by sprays. Where 3 pounds of nitrogen came from manure alone, the yields were no greater than where only half as much was used and the remaining nitrogen came from urea.

In both sand- and solution-culture experiments, carried on over a four- to ten-year period with young navel orange trees, the author (Chapman and Liebig, 1942, unpublished data; and Chapman *et al.*, 1955, unpublished data) found no evidence of yield decreases from excessive amounts of nitrogen (as nitrate). Burkhart (1959), in field experiments with young Lisbon lemons on rough lemon rootstock on a sandy soil, got best yields from the use of about 3½ pounds of nitrogen per tree per year. Part of the nitrogen came from manure, and phosphate fertilizer was added. Less nitrogen decreased yields, and more nitrogen (about 5 pounds) produced close to the same yield as the 3½-pound rate.

Embleton *et al.* (1959), comparing the yields where nitrogen was omitted for five years with a "ranch treatment" (which provided about 1 pound nitrogen per tree per year) on an old Valencia orchard planted on a deep Yolo loam soil, found that the residual nitrogen from previous years of fertilization was sufficient to meet tree needs. The additional nitrogen (over the 1 pound of "ranch treatment") did not decrease yields, neither did it increase yields. Leaf analyses made for three of the years of the experiment showed 2.22, 2.32, and 2.32 per cent nitrogen in the dry matter of the "no nitrogen" plots as compared to 2.51, 2.37, and 2.36 per cent nitrogen in the "ranch-treatment" trees.

Various California surveys (summarized by Platt, 1960) showed that where yield was related to nitrogen usage, greatest yields were on orchards receiving the highest nitrogen rate. For example, in the early 1920's a survey of 1,000 orchards showed highest yields where 350 pounds of nitrogen per acre per year were used.

Reitz and Hunziker (1962) conducted a nitrogen-rate experiment on native Marsh grapefruit budded to sour orange rootstock on a hammock soil in the Indian River area of Florida. Best yields (six-year average) and size were obtained by adding 2 pounds of nitrogen per tree per year. Four pounds of nitrogen per tree slightly decreased yields and sizes and coarsened the fruit somewhat. Analyses of leaves from spring-flush, nonfruiting terminals picked in July showed an average of 2.13 per cent of nitrogen at the 2-pound nitrogen rate, 1.70 per cent with no nitrogen, and 2.25 per cent at the 4-pound nitrogen rate. Stewart, Leonard, and Wander (1962), in a five-year experiment with Pineapple oranges on rough lemon rootstock found that best yields with ammonium nitrate were at rates of 100 to 200 pounds of nitrogen per acre per year, while 550 pounds markedly decreased the yield. They suggested that the high rate of fertilizer might have solubilized enough copper to be toxic. With sodium nitrate, best yields were at a rate of 350 pounds nitrogen per acre per year. Smith and Rasmussen (1961) found with Marsh grapefruit that 540 pounds of nitrogen per acre annually did not decrease yield, as compared with rates of 120 and 240 pounds of nitrogen.

Quoting from a general discussion on fertilization of citrus in Florida, Smith (1959) says: "In Florida, nitrogen has little direct effect on fruit quality over a fairly wide range of rates and sources. Indirectly it has greatly improved fruit quality, as ample use of this element is the most important nutritional factor in gaining a good set of fruit. As just indicated, a good set of fruit is essential for high quality fruit. The average grove should receive 150 to 200 pounds of nitrogen annually per acre. The consensus of technical opinion indicates that 0.4 pounds of nitrogen per box of fruit is still an appropriate guide for orange production in most cases, but experimental work now shows that this is excessive for grapefruit and the recommendation has been lowered to 0.3 pounds for this variety."

Bouma and McIntyre (1963), reporting on orange yields from 1950 to 1960 in a field experiment in the Murrumbidgee irrigation area of New South Wales, Australia, indicated that maximum yields were obtained in noncultivation plots (weeds controlled by kerosene sprays) at a nitrogen rate of 1.6 pounds per tree. Yields were about as good with 0.8 pound of nitrogen and were depressed at 3.2 pounds of nitrogen. The source of nitrogen was ammonium sulfate, and at the highest nitrogen rate soil acidity was reduced to pH 4.5. Reduced phosphorus availability may have accounted for poorer yields and fruit quality at the high nitrogen levels. The low pH of the soil may also have produced other detrimental effects.

A six-year experiment with fourteen-year-old Shamouti oranges (on sweet lime stock) on a loamy sand at Rehovot, Israel, carried on by Oppenheimer and Heymann-Herschberg (1954), produced maximum yields at rates of about 200 pounds of nitrogen per acre, though yields were only slightly less with half this amount and but slightly decreased at the 300-pound rate. The six-year average per tree for three nitrogen rates of 100, 200, and 300 pounds of nitrogen per acre were 97.8, 104.4, and 101.3 kgm of fruit per tree, respectively. Ammonium sulfate was used as a nitrogen source and all trees received, in addition, both superphosphate and potash. In spite of this, available soil phosphate was reduced by the heaviest ammonium sulfate application, but leaf phosphate was not affected. During the last year of the experiment, nitrogen in spring-flush leaves from fruiting terminals showed 2.59, 2.87, and 2.82 per cent of total nitrogen in the dry matter of the leaves for the three nitrogen rates, respectively. Fruit size was not affected, but commercial quality was decreased by the high nitrogen treatment. This may have been due to lack of sufficient phosphate.

Singh and Agrawal (1959, 1960) carried on a nitrogen-rate experiment with several varieties of oranges and mandarins (all on Karna Khatta rootstock). The soil was a loam to clay loam. Differential nitrogen applications using ammonium sulfate were begun when the trees were only two years old, and four rates were used. At the outset, the respective rates per tree were 2.3, 3.6, 6.0, and 9.6 ounces of actual nitrogen for the N_1, N_2,

N_3, and N_4 treatments. When the trees were six years old, the respective rates were 12, 18, 30, and 48 ounces of nitrogen per tree. The average cumulative yields on the four orange varieties for the last four years of the experiment were 36.8, 44.3, 81.0, and 79.5 kgm of fresh fruit per tree for the N_1, N_2, N_3, and N_4 treatments, respectively. The percentages of juice, total solids, citrus acid, and vitamin C were only very slightly affected. The average last four-year yields of the mandarin varieties were 57.8, 72.1, 78.9, and 86.1 kgm per tree for the N_1, N_2, N_3, and N_4 treatments, respectively. The N_3 treatment had the highest yield of juice.

Reuther and Smith (1950, 1952) conducted a Florida field experiment involving three rates of nitrogen, potassium, and magnesium with five-year-old, bearing Valencia oranges on rough lemon stock on a sandy soil. They obtained best yields at the highest nitrogen rate (3 pounds of nitrogen per tree). Fruit size decreased slightly, as did total soluble solids and vitamin C at the high nitrogen rate; total acid increased slightly, and degreening of the rind and maturity of the fruit was slightly delayed. Spring-flush (March) leaves from fruiting terminals picked in December showed 2.30, 2.48, and 2.65 per cent nitrogen, respectively, in the dry matter.

In an outdoor, sand-culture experiment with Valencia oranges on rough lemon rootstock, involving three levels of nitrogen (30, 80, and 210 ppm of nitrogen in the nutrient solution), potassium, and magnesium, Smith and Reuther (1953) reported best tree growth and yields at the highest nitrogen levels. Fruit size was somewhat decreased, as was vitamin C, but total juice, soluble solids, and acid were increased. Rind thickness was not appreciably different at the three nitrogen rates.

Maxwell and Dacus (1958) applied ammonium nitrate, along with other nutrients, to red grapefruit under sod culture in Texas. They found that only nitrogen influenced yields and that fruit quality was more affected by season than by treatment.

Rosselet, Helff, and Langenegger (1962), reporting on a 20-year experiment with Valencias on rough lemon rootstock in South Africa, found that highest yields resulted from the use of ammonium nitrate plus manure. During the last five years of the experiment, this treatment, which supplied 4.25 pounds of nitrogen per tree per year, produced an average of 651 pounds of fruit per tree as against 579 pounds from ammonium nitrate alone at a rate of 2.3 pounds of nitrogen. A control treatment receiving no nitrogen produced an average of 196 pounds of fruit. All plots received a uniform application of phosphorus, potassium, and magnesium fertilizers and were regularly treated with a zinc-copper spray. Fruit quality was not materially affected by the higher nitrogen rate. Rind thickness for the control, ammonium nitrate (NH_4NO_3), and NH_4NO_3 plus manure, respectively, was 0.39, 0.53, and 0.50 cm; total solids 10.7, 11.1, and 11.2; percentage juice 55.0, 52.0, and 54.0; and per cent acid 1.24, 1.22, and 1.41.

No leaf analysis values were reported. The authors ascribed the high yields on the NH_4NO_3 plus manure plot over NH_4NO_3 alone to the extra nitrogen. The soil in the experiment was a reddish, lateritic-type sandy loam, well drained and permeable. This experiment showed that in the absence of other limiting factors high rates of nitrogen fertilizer produced high yields with no impairment of fruit quality.

In contrast to these results, a seven-year South African fertilizer experiment with young navels on rough lemon rootstock planted in 1952 (differential fertilizer treatments started in 1956) on a deep-reddish sandy loam, which had had several croppings to cowpeas preceding the citrus, gave no response to nitrogen over a basic 1 pound of nitrogen per tree per year rate (Bester, 1965). This experiment consisted of sixty-four treatments involving nitrogen, phosphorus, potassium, calcium, magnesium, and manure in all combinations. Average yields per tree for the last five years of the experiment (1959–63) and leaf analysis values on nine- to ten-month-old spring-cycle leaves from fruiting terminals were as follows:

Treatment	*Yield in Pounds of Fruit per Tree, 5-Year Average, 1959–63*	*Per Cent Total N in Leaves in 1963*
Control (no nitrogen)	198.0	2.20
Nitrogen	257.6	2.51
Nitrogen and phosphorus	317.3	2.61
Nitrogen, phosphorus, and manure	307.5	3.20
Nitrogen, phosphorus, and potassium	350.1	2.50
Nitrogen, phosphorus, magnesium, and manure	366.9	2.97

There were significant yield responses to phosphorus as well as nitrogen in the experiment,

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but none to potassium. The manure added 1.72 pounds of nitrogen in addition to the 1 pound from the NH_4NO_3.

Lack of yield responses to extra nitrogen in the experiment is probably due (1) to the trees being young—eleven years from planting at the end of the experimental period; and (2) very low leaching losses of nitrogen owing to the careful water-sparing irrigation practices used and low effective rainfall in the area. No fruit quality information was reported in Bester's thesis, although quality data was obtained.

In long-term experiments with Washington navel oranges in the San Joaquin Valley of California, Jones and Embleton (1967) found no yield response to nitrogen beyond an annual application of one pound per tree. They noted also that deleterious effects on fruit quality were obtained with increasing rates of nitrogen application, and that Washington navel oranges have about the same minimum adequate level of nitrogen in leaves as in Valencia oranges.

The review of literature above is sufficient to indicate that in many, though not all, cases increased nitrogen has resulted in increased yield. Yield decreases, where they have occurred, are often related to adverse effects on the availability of other nutrients (notably phosphorus), or the release of toxic amounts of elements such as copper. On the other hand, fruit sizes and external and internal quality factors may be slightly impaired under some conditions, but this is not universal.

As for the secondary or side effects of nitrogen on soil reaction, nutrient availability, soil structure deterioration, nitrite accumulation, biuret, and potassium perchlorate toxicity, these may stem from the forms, rate, timing, and manner of application; soil characteristics, rootstocks, and varieties also are involved.

In addition to the well-known effects of different nitrogen fertilizers on soil reaction and the whole chain of changes these may evoke in nutrient availability, there are the more or less immediate changes in the soil solution resulting from the cation or anion with which the nitrogen is associated. The nitrification of organic and ammonium forms of nitrogen usually makes for temporary increases in the calcium (and to a lesser extent magnesium) content of the soil solution, and large increases in these cations may decrease the phosphorus, boron, zinc, manganese, and copper intake by plant roots and, in some soils, actually aggravate deficiencies of these elements. Where excess boron is a problem, nitrogen fertilization sometimes decreases boron toxicity (Cooper, Peynado, and Olson, 1958; Jones *et al.*, 1963).

The effects of various nitrogen fertilizers on the micronutrients of citrus leaves have been studied by Labanauskas *et al.* (1958*b*). Where soils had become more acid from the use of ammonium nitrate and sulfate, manganese absorption was increased. On the other hand, calcium nitrate decreased manganese absorption. All three fertilizers reduced boron absorption, but none of them affected zinc and iron absorption.

In special circumstances (e.g., acid, sandy soils), heavy applications of ammonium fertilizer may result in considerable temporary accumulation and uptake of the ammonium ion and depression of manganese, magnesium, and potassium absorption.

Soil structure and related changes (water penetration, salt accumulation, and tree deterioration), resulting from the continued use of sodium nitrate and ammonium sulfate at rates giving 3 pounds of nitrogen per tree per year, have been described by Aldrich, Chapman, and Parker (1944); Aldrich, Parker, and Chapman (1945); Jones, Cree, and Embleton (1961); and Lombard *et al.* (1962). Smith (1962) found that Valencia orange trees on rough lemon roots were injured by sodium nitrate applied at rates of 285 and 330 pounds of nitrogen per acre per year. The injury was due to excessive sodium absorption.

Aldrich *et al.* (1944) found that the sodium from sodium nitrate partially displaced calcium from the exchange complex in the surface of the soil, decreased permeability to water, and resulted in salt accumulation, moisture deficiency, and tree deterioration. The ammonium sulfate treatments made the soil so acid that ammonium, instead of nitrifying, accumulated in the exchange complex, causing decreased permeability to water and increased salt accumulation. This treatment thus produced the same effects on the trees as sodium nitrate.

The accumulation of nitrite in soils was thoroughly explored by Chapman and Liebig (1952). In alkaline soils, they found especially high accumulations of nitrite from the use of urea. In winter periods, when the soil is cold, this constituent sometimes persisted in soils for several months before it was converted to nitrate. Under acid soil conditions, no nitrite accumulated. The apparent explanation is that under alkaline soil

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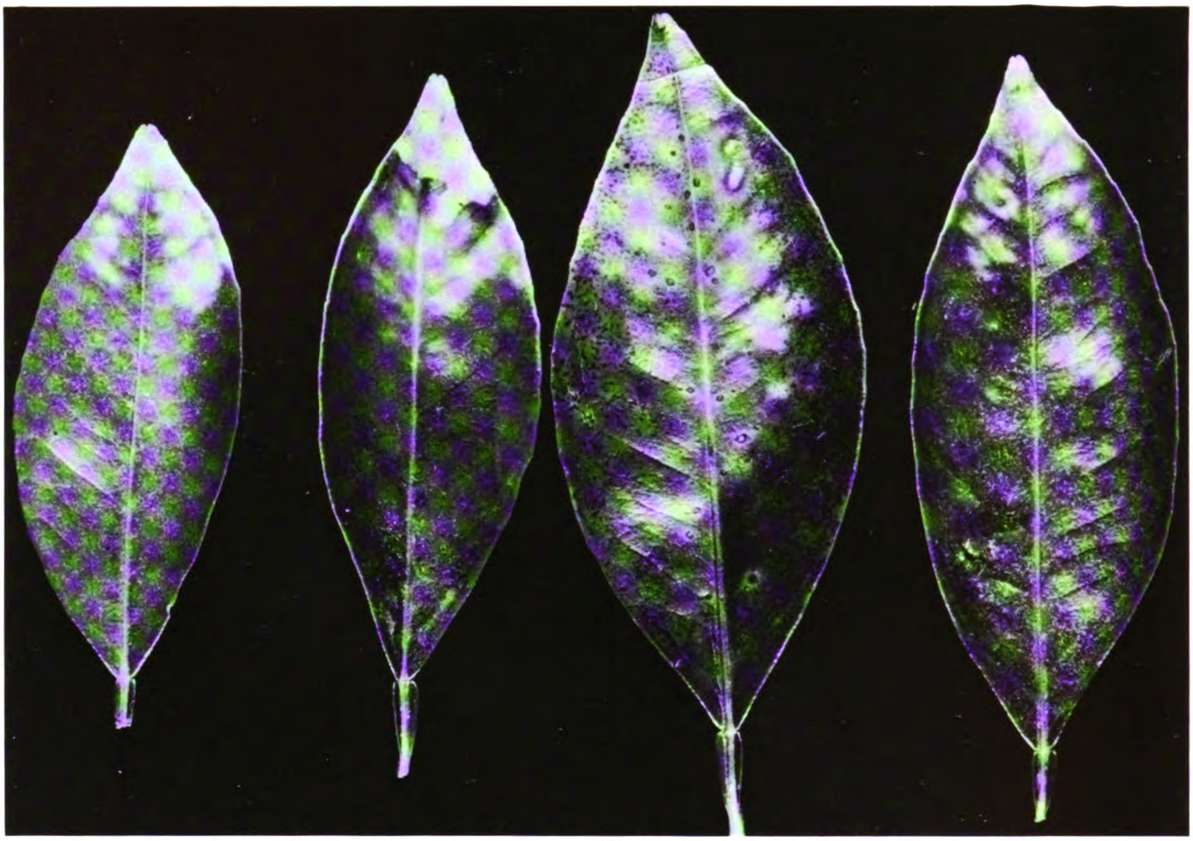

Fig. 3–38. Type of apex mottling and necrosis caused by excess biuret absorption.

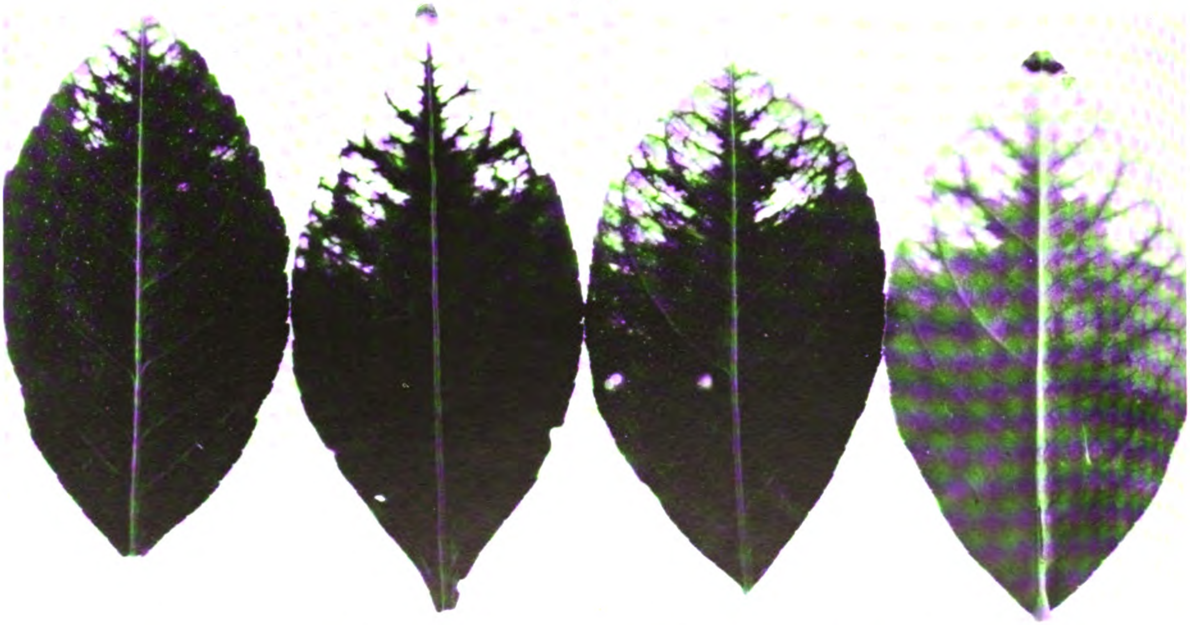

Fig. 3–39. Apex mottling caused by potassium perchlorate impurity in Chilean sodium nitrate.

conditions nitrobacteria convert nitrite to nitrate less rapidly than ammonium is converted to nitrite, and thus the latter accumulates. No damage to the citrus trees were observed in plots where up to 90 ppm of nitrogen as nitrite accumulated. Bingham, Chapman, and Pugh (1954) found that nitrite was much less toxic under alkaline than under acid soil conditions. Since no nitrite has been encountered under the latter conditions in arable soil, it appears that nitrite is not likely to be a problem except under such special circumstances as anaerobic conditions. In a study of silica-gravel cultures to which urea was added, Bitcover and Wander (1950) found that nitrite accumulated in the warm period of the year. The trees wilted in cultures where up to 23 ppm of nitrogen as nitrite was found.

The toxicity of biuret ($NH_2CONHCONH_2 \cdot H_2O$) to citrus has been investigated by Jones (1954), Impey and Jones (1960*b*), Haas and Brusca (1954*a*), Iwasaki and Shichijo (1962), and others. Biuret is formed when urea is heated. Its toxicity to pineapple plants was reported by Sanford *et al.* (1954).

The yellow-tip mottling effects produced on citrus leaves by biuret are illustrated in figure 3–38. Biuret causes permanent injury and the leaves fall prematurely. Injury can result from either foliar or soil application of urea containing biuret in amounts varying from 0.5 to 2.5 per cent or more. This compound accumulates, as such, in the apex of citrus leaves and appears not to be metabolized, according to Impey and Jones (1960*b*). They found somewhat less protein in the injured leaf tips and an increase in several amino acids, especially glycine and serine.

The biuret problem has been largely overcome by the production of urea containing < 0.25 per cent of this compound and by using urea (in spray formulations) at rates not exceeding 7.5 pounds to 100 gallons of water.

Potassium perchlorate injury is illustrated in figure 3–39. It was widely observed in citrus in California and elsewhere during the World War II years, when Chilean sodium nitrate was exclusively used. It occurs as an impurity in this salt. In the early stages of leaf injury, it appears not to adversely affect tree health or production, but severely injured leaves drop prematurely and there are thought to be some adverse effects on production.

This brief review of nitrogen excess problems is sufficient to indicate the diversity of troubles which may ensue from the overuse of nitrogen and some of the difficulties for which to watch. Leaf and soil analyses are powerful tools to help guide fertilizer practices. It is not enough, however, to merely check citrus leaves for nitrogen content alone. Periodically, complete leaf analyses are needed and adjustments of fertilizer and management programs must be made. As the secondary ill effects of nitrogen fertilization are corrected, higher yields can often be obtained from extra nitrogen fertilizer additions with increased profits to the grower.

Control of Nitrogen Excess.—Fortunately, the ill effects of overuse can be avoided or easily corrected. Decreases in nitrogen use, or changes to other forms, correction of soil acidity or alkalinity, use of gypsum or organic materials to improve soil structure, and foliar sprays to correct nutritional deficiencies are some of the self-evident measures which can be put into use when it is determined that the form, amount, timing, or manner of application of nitrogen fertilizer is causing harmful effects.

PHOSPHORUS (P)

Phosphorus Deficiency

Before World War II, phosphorus deficiency of citrus had not been positively identified in the field. Knowledge of its effects on citrus was based

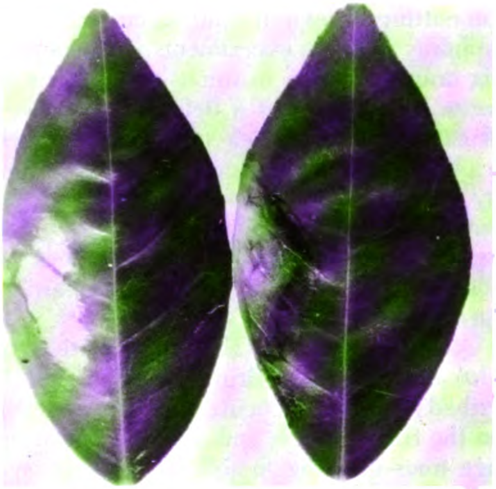

Fig. 3–40. At time of spring growth and blossom, old leaves from phosphorus-deficient trees commonly show large necrotic areas similar to burning from excess salt. The necrotic areas may be at the tip, on the margins, or at other places on the leaves. Such leaves are usually faded and may show slight vein chlorosis.

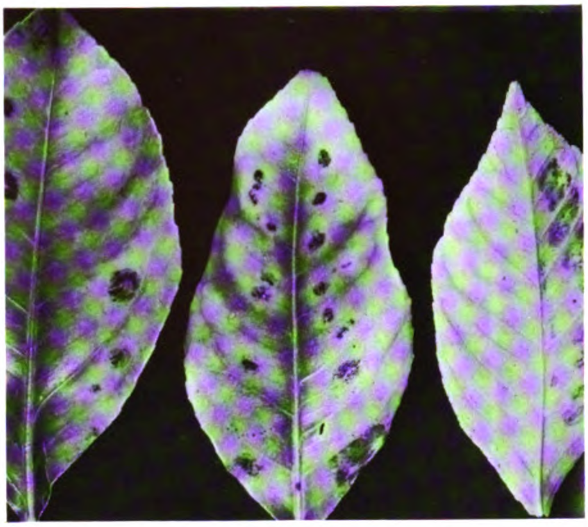

Fig. 3–41. Brown spots on upper sides of lemon leaves caused by phosphorus deficiency. The older leaves are the ones affected by this spotting, and after this condition occurs they absciss prematurely. Usually lemon trees exhibiting this brown spotting tend to show lack of vigor, but this condition has sometimes been noted on lemon trees of decided vigor. Such leaves are very low in phosphorus content, usually well under 0.10 per cent in the dry matter.

on the symptomatology produced on young citrus plants and trees grown in sand, soil, and solution cultures. Haas (1936*b*) reported experiments with lemon cuttings grown in solution cultures lacking phosphorus and also experiments with young Valencia oranges grown in minus-phosphorus sand cultures. He found that a deficiency of this element caused the older leaves of both species to turn a brownish-green color and fall prematurely. Many leaves developed water-soaked and, later, necrotic areas on the margins or tips (fig. 3–40). Phosphorus-deficient leaves showed values ranging between 0.04 to 0.07 per cent of phosphorus in the dry matter.

Subsequently, Chapman and Brown (1941*a*) and Chapman, Brown, and Liebig (1943) described the effects of acute phosphorus deficiency on the fruit, foliage, and composition of navel orange trees growing in soil. The foliage symptoms were similar to those reported by Haas. The fruit produced on the phosphorus-deficient trees was rather coarse, had thicker rinds, and had a lower juice content than fruit from trees well supplied with this element. The acid was higher, but total solids in the juice were not measurably affected. Analyses of various parts of healthy and phosphorus-deficient trees showed very low levels of phosphorus in all parts of the plant except the young leaves. The old leaves of the phosphorus-deficient trees contained 0.05 per cent total phosphorus as compared with 0.11 per cent in comparable leaves from healthy trees. Nitrogen, potassium, and magnesium were higher in the phosphorus-deficient leaves, but calcium was lower.

Still later, Chapman and Rayner (1951) published the results of a nine-year experiment with orange trees growing outdoors in nutrient solutions of graded phosphorus content. The effect of phosphorus lack on growth, behavior, yield, fruit size, fruit quality, and inorganic composition confirmed all of the previously mentioned observations and added much additional information. Of special interest was the finding that the very earliest effect of low phosphorus was on fruit quality. A greater percentage of the fruit from such trees lacked firmness, showed pith separation, and had thicker rinds and less juice than the fruit from trees getting more phosphorus. These findings coincided with studies of fruit quality emerging from field experiments reported from various parts of the world: Takahashi (1931), Morris (1937), van der Plank and Turner (1936), Anderssen (1937), Anderssen and Bathurst (1938), Crous (1937), Allwright (1938), Esselsen and Oberholzer (1939), Bathurst (1945), Innes (1946), Forsee and Neller (1944), Hilgeman (1941), Finch and McGeorge (1945), Jones and Parker (1949*a*), Smith, Reuther, and Gardner (1949), Young and Forsee (1949), and Winnik (1950). Much of the literature cited above was summarized by Chapman and Rayner (1951) and need not be repeated here.

Up to 1949, the only field experiment in which yield increases from phosphorus had been reported was that of Forsee and Neller (1944). They found that Valencia oranges growing on an acid peat soil in Florida benefited from phosphorus. Trees in a plot not receiving phosphorus were smaller, achieved less vegetative growth, and had smaller and somewhat narrower leaves. The fruit from phosphorus-deficient trees was coarse, soft, bright orange in color, thicker in rind, and frequently misshapen; the interior structure of the fruit was coarse, and the juice was more acid. There was a greater preharvest drop of fruit from the trees lacking phosphorus than from those receiving phosphorus fertilizer. The high-phosphate treatment produced a greater percentage of am-

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Table 3–27

PRINCIPAL PHOSPHORUS DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Phosphorus Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Excessive abscission of old leaves during and after spring blossom.	1. Potash deficiency leads to excessive leaf fall after bloom in the spring, but this is not of invariable occurrence.
2. Burned areas at the tips or sides of many old leaves which fall during spring-blossom cycle (see fig. 3–40).	2. Necrotic areas in leaves are not seen in the spring. Salt, boron excess, and other nutritional disorders can cause this symptom in the fall.
3. Bronze, lusterless coloration of older foliage; in such leaves phosphorus content is low, and nitrogen is high.	3. Nitrogen deficiency can cause similar lusterless, faded coloration, but, in this case, total nitrogen will be low and phosphorus high.
4. Sparse bloom and weak new spring-cycle growth (see fig. 3–42).	4. Sparse bloom is not an uncommon cyclic condition in citrus, especially on varieties susceptible to alternate-bearing tendency.
5. Some dieback in the spring of weakened twigs.	5. Dieback is common to potassium, boron, calcium, and other deficiencies and to salt excess.
6. Reduced growth of tree and thin foliage in more acute stages; however, some new terminal growth occurs periodically, despite devitalized condition of the tree. Trees are more susceptible to leaf drop and dieback from wind and high temperatures.	6. Many other conditions can cause devitalization and loss of foliage.
7. Mild manganese deficiency leaf patterns may occur as a secondary condition.	7. Manganese deficiency.
8. Large, irregular, bronze-colored gum excrescences occur on the upper surfaces of lemon leaves (see fig. 3–41). Orange leaves show this condition less commonly.	8. No other condition is known which produces this same symptom.
Fruiting and Fruit Characteristics	
1. Yield is markedly reduced when deficiency reaches moderate stage.	1. Most other nutrient deficiencies reduce yield.
2. Maturing fruit becomes prematurely soft and spongy, center pith separates, and segments separate in more advanced stages.	2. No other condition is known which produces this effect, though extreme drought may cause fruit to lose turgor.
3. Abnormal preharvest drop of fruit.	3. Abnormal heat spells and insecticide sprays can sometimes cause excessive fruit drop at early stages of maturity.
4. Rinds are somewhat thicker and coarser than usual for the variety, and there is less tendency to creasing.	4. Boron, sulfur, zinc, and calcium deficiencies, as well as certain climatic factors, can cause increased rind thickness.
5. Sizes may be slightly increased, probably due to decreased yield.	5. Any factor which decreases yield greatly is likely to result in larger-sized fruit.
6. Orange color of rind may deepen.	6. Rind color is markedly affected by climate; cool nights of desert areas tend to deepen color.
7. There is a tendency for misshapen fruit.	7. Boron, calcium, copper, and sulfur deficiencies often cause misshapen fruit.
8. There is less juice and more pulp.	8. Excess potash, lack of sulfur, and boron can produce similar effects.
9. Juice acidity is increased.	9. High potash increases juice acidity.
10. Vitamin C is increased.	10. High potash will increase vitamin C slightly.

moniation (copper deficiency in the fruit). Total phosphorus in the leaves of the phosphorus-deficient trees was 0.07 as compared with 0.098 per cent in the dry matter in trees receiving a 3–12–12 fertilizer. The phosphorus content of the fruit juice was 0.89 compared with 3.46 ppm in the deficient and amply supplied treatments, respectively. In the soil, an acid extract showed 8 versus 94 pounds of phosphorus per acre for the two plots. A more recent account of this work was given by Young and Forsee (1949).

In 1949, a lemon tree condition noted in California, characterized by a brown-leaf-spot splotching on older leaves (fig. 3–41) and often accompanied by poor tree condition, was diagnosed by Aldrich and Haas (1949) and Aldrich and Coony (1951) as being due to phosphorus deficiency. Leaves thus affected showed total phosphorus levels in the deficiency range, as established by Chapman and Brown (1941*a*) and Chapman (1949). Responses to phosphate injection into the tree or applications to the soil were dramatic. Affected lemon trees were found in several parts of California, and a few orange orchards were also discovered which showed phosphorus deficiency symptoms. Subsequently, Embleton, Kirkpatrick, and Parker (1952) and Embleton *et al.* (1956) found an orange orchard with many of the same fruit and foliar symptoms, and leaf analysis values were in the phosphorus deficiency range. Marked responses to phosphorus fertilization were obtained. Phosphorus deficiency in this case had been brought about by the continued use of ammonium sulfate on a soil already low in total phosphorus.

Heymann-Herschberg (1952) in Israel, reporting on phosphate trials of several Shamouti orange groves, found indications of a yield response in one grove and noted fruit size and quality effects consistent with those reported by others; in several groves, phosphate decreased yield, apparently because of an antagonistic depression of nitrogen absorption.

In a Valencia orange (on rough lemon rootstock) field experiment at Nelspruit, South Africa, carried on from 1942 to 1956 with trees planted in 1934, Rosselet *et al.* (1962) got marked yield responses to phosphate fertilizer. Fruit rind thickness was decreased, percentage of juice increased, juice acidity decreased, and total soluble solids slightly decreased in the fruit from phosphate-fertilized trees. Phosphorus deficiency has also been noted in a few other places in South Africa (DeVilliers *et al.,* 1958). Bester (1965) has recently reported yield increases from phosphate in the soil of the Zebediela estate plantings in South Africa. Bouma (1959) and Bouma and McIntyre (1963) reported marked yield and fruit quality improvements from phosphate in the nontillage plots of a Valencia and navel orange fertilizer experiment carried on in the Murrumbidgee irrigation of New South Wales, Australia. The plots had been given variable irrigation and nitrogen treatments (as ammonium sulfate) beginning in 1947. By 1950, evidence of phosphorus lack became apparent. From that time on, yearly applications of superphosphate were made. There was a marked improvement in yield and fruit quality.

In Jamaica, Innes (1946), reported yield responses to phosphate. Workers in Brazil also have stated that phosphorus is needed on many of their soils.

Based on an extensive series of soil analyses, pot-culture tests, a review of the earlier California phosphate experiments (Chapman, 1934), and a subsequent leaf-analysis survey by Chapman and Fullmer (1951*a*), it was concluded that very few commercial citrus orchards in California were in need of supplemental phosphorus. This is due in part to large accumulations of phosphate from the past use of manure and mixed fertilizers, coupled with the fact that the natural supply of phosphorus in many California citrus soils is good. Also, the phosphorus requirement of citrus is not high as compared with that of many quick-growing field and vegetable crops. However, it is necessary to be on the alert for phosphorus deficiency in all citrus-growing areas. Periodic leaf analyses should be made and fruit quality checked for deficiency symptoms.

Effects of Phosphorus Deficiency.—As a result of the aforementioned nutritional work and field fertilizer trials, a good understanding of the effects of phosphorus lack on tree appearance, growth behavior, leaf symptoms, and fruit quality is now at hand. Moreover, the leaf tissue content of phosphorus and other inorganic nutrients which characterize phosphorus deficiency have been established. In addition, reasonably good soil analysis criteria for detecting phosphorus deficiency of citrus are available.

As with boron, potassium, nitrogen, and other deficiencies, there is no one specific leaf pat-

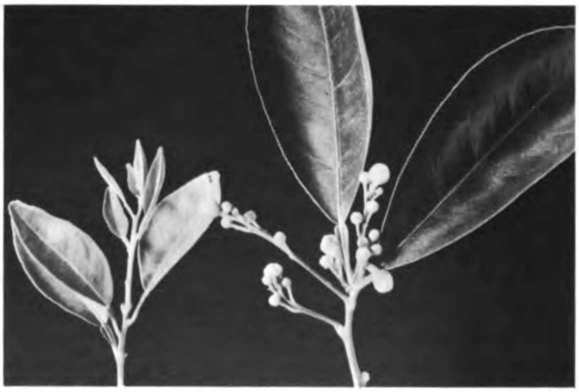

Fig. 3–42. Orange shoots showing weak spring growth and lack of bloom on phosphorus-deficient tree (left), as compared with larger leaves and abundant bloom on tree with ample phosphorus (right). Old foliage of phosphorus-deficient tree is lusterless and faded, and the leaves are small.

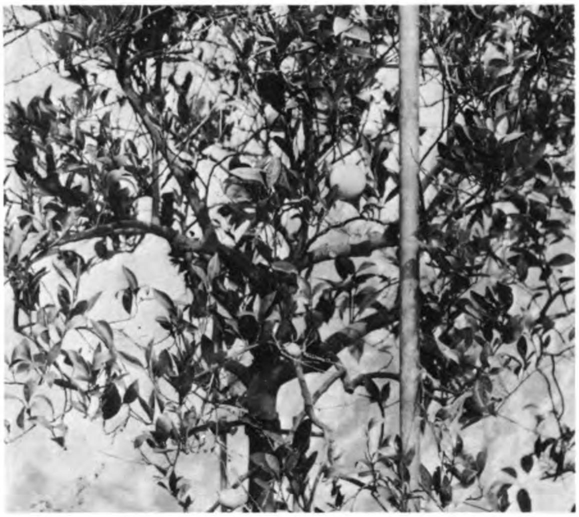

Fig. 3–43. Navel orange tree showing effects of acute phosphorus deficiency. Foliage becomes thin, leaves become faded and bronzed, there is twig dieback, new growth is sparse, yield is greatly decreased, and fruit is of poor quality, but sizes are not especially decreased; often sizes are increased because of low yields.

tern or condition which of itself signifies clear and unmistakable evidence of phosphorus deficiency. However, the combination of foliar, growth, and fruit characteristics (supplemented by leaf and soil analyses) makes possible an almost certain diagnosis when the deficiency is moderate to acute. The range of foliar growth, yield, and fruit effects produced by lack of phosphorus is recorded in table 3–27.

In the moderate to acute stages of phosphorus deficiency, there is premature abscission of old leaves, especially heavy in the spring following bloom; many of these old abscising leaves may show burned areas, either at the tip or elsewhere on the leaf (fig. 3–40). New growth is sparse and weak, and bloom is commonly markedly reduced (fig. 3–42). Weakened shoots die back, and the net effect of the reduced growth is to produce a thinly foliated tree, with much dead wood in evidence (fig. 3–43). The foliage lacks luster, and many leaves take on a faded-bronze appearance similar to that characterizing nitrogen deficiency. In the case of lemon trees, it is common to find many old leaves which show irregularly rounded, brown spots on the upper surface (fig. 3–41). Such leaves abscise prematurely.

Not only is fruit set markedly curtailed, but there is commonly a marked preharvest drop in cases where the deficiency has not yet become acute.

The maturing fruit lacks firmness, has somewhat thickened rinds, and some of the fruits are misshapen. There is a center separation of pith, and sometimes the segments are separated a little. The fruit is rather soft and spongy and of low juice content (figs. 3–44 and 3–45). Maturity is delayed and comparison at any given time shows

Table 3–28

TOTAL PHOSPHORUS (IN DRY MATTER) IN SPRING-CYCLE LEAVES COLLECTED AT MONTHLY INTERVALS FROM TREES GROWING IN SOLUTIONS OF VARIOUS PHOSPHATE LEVELS

Month	Age of Leaves (Months)	Phosphorus-Deficient Trees (Per Cent)	Slightly Phosphorus-Deficient Trees (Per Cent)	Trees with Medium Phosphorus Supply (Per Cent)	Trees with High Phosphorus Supply (Per Cent)
May	1	0.061	0.080	0.103	0.132
June	2	0.072	0.105	0.127	0.148
July	3	0.073	0.095	0.131	0.155
August	4	0.077	0.107	0.127	0.149
September	5	0.082	0.117	0.138	0.161
October	6	0.087	0.113	0.135	0.155
November	7	0.094	0.119	0.139	0.158
December	8	0.090	0.114	0.137	0.159
January	9	0.090	0.115	0.137	0.169
February	10	0.082	0.105	0.122	0.158
March	11	0.067	0.089	0.114	0.137
April	12	0.058	0.074	0.098	0.127
Average		0.080	0.100	0.130	0.150

Source: Chapman and Rayner (1951).

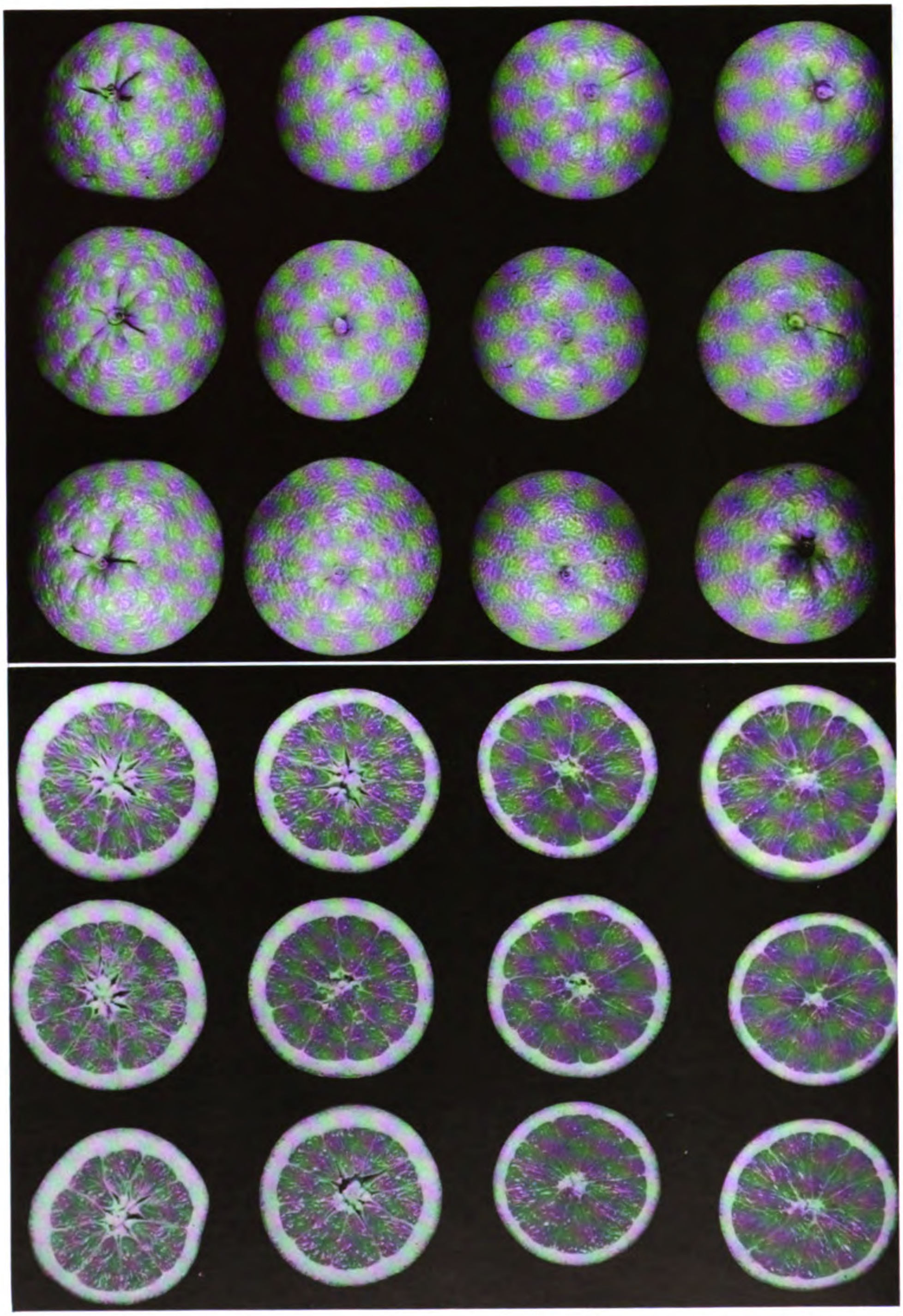

Fig. 3–44. Effect of increasing phosphate supply (from left to right) on exterior (top) and interior (bottom) characteristics of fruits. The three fruits at the left are from a tree acutely deficient in phosphorus; the next three fruits are from trees showing no visible signs of deficiency, but characteristics of some of the fruits are like those from acutely deficient trees. The low phosphorus fruits have a slightly deeper color, the sizes are a little larger, and the fruits are more misshapen, somewhat coarser, have thicker rinds, and the centers are separated. The last two rows of fruits are from trees receiving an ample supply of phosphorus.

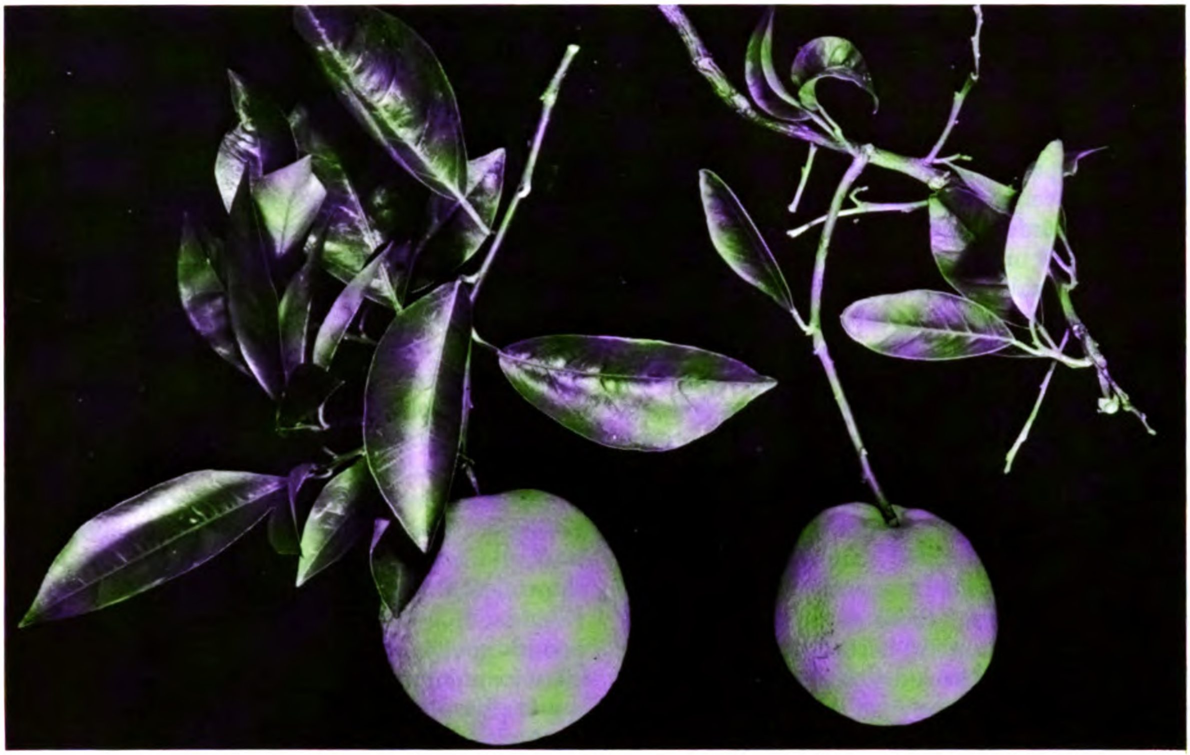

Fig. 3–45. Fruit-bearing twigs from phosphorus-deficient (right) and normal (left) navel orange trees. Fruit on phosphorus-deficient twigs is somewhat misshapen and spongy. There is also considerable defoliation. Fruit from normal twigs is more regular in shape and is firm.

a higher acid and vitamin C content than with fruit not lacking in phosphorus. The color of phosphorus-deficient fruit is often a deeper orange than that of fruit not lacking phosphorus. Fruit size is usually somewhat increased where phosphorus is lacking; this is probably due in part to less fruit on the tree.

Early Symptoms of Phosphorus Deficiency.—In the carefully controlled water-culture experiment reported by Chapman and Rayner (1951), the earliest evidence of phosphate lack was denoted by fruit symptoms only. The trees themselves showed no obvious foliar or growth symptoms (fig. 3–46). Foliage was dark green and abundant, new cycle growth was abundant, bloom was normal, and yields of fruit were good. However, some of the fruits from these trees were spongy, lacked firmness, and had hollow centers. These conditions characterized a high percentage of fruit from trees moderately or acutely deficient in phosphorus. Where such conditions are encountered, supplemental leaf and soil analyses should be made, and, if these values are on the low side, trials with phosphate fertilizer should follow.

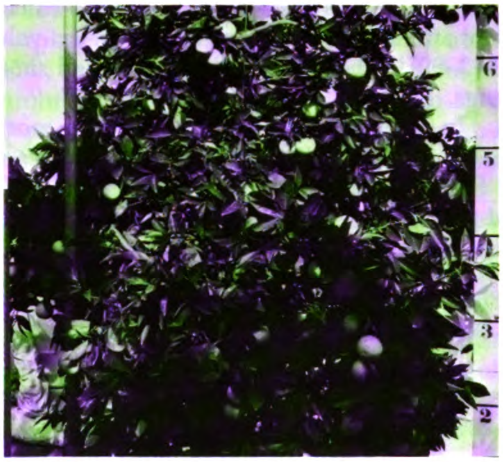

Fig. 3–46. Navel orange tree showing foliage and fruit in early stage of phosphorus deficiency. At this stage, there are no apparent foliage or growth symptoms. The foliage color is dark green, and the yield is not impaired, but some of the fruits display such abnormalities as separated centers, thick rinds, and inner pulpiness. When the deficiency is more advanced, a much higher percentage of fruits show these symptoms.

Leaf Analysis Values.—Although there are seasonal trends in the per cent of total phosphorus in the dry matter of leaves, the phosphorus content of leaves from trees not lacking this element usually show 0.10 per cent or more of total phosphorus at all seasons. On the other hand, three- to seven-month-old leaves from phosphorus-deficient trees usually show values of less than 0.10 per cent of phosphorus. Table 3–28 shows the monthly values for total phosphorus in spring-cycle leaves picked from bearing trees growing in solutions of graded and maintained phosphorus content (Chapman and Rayner, 1951). It will be noted that the leaves from trees acutely deficient in phosphorus were always under 0.10 per cent; the leaves from trees showing the very earliest stage of phosphorus deficiency showed values under 0.10 per cent *only* in the spring months; whereas, trees amply supplied rarely showed values under 0.10 per cent. Leaf analyses reported by Aldrich and Coony (1952) and Embleton *et al.* (1952) show total phosphorus values in orange leaves from phosphorus-deficient trees below 0.10 per cent except in young April-picked leaves. With the May sampling, however, the orange leaf values had dropped to 0.08 per cent of phosphorus, and they remained below the 0.10 per cent value for the remainder of the year. Associated with the low phosphorus content of leaves from phosphorus-deficient trees is a lower-than-average calcium content and higher nitrogen and potassium values (table 3–29). Other workers have reported similar findings.

In addition to the analyses already reported here, Haas (1936*a*, 1947) has reported many analyses for phosphorus in fruit, flowers, and other plant parts.

Table 3–29

EFFECT OF VARYING PHOSPHATE SUPPLY ON INORGANIC COMPOSITION OF ORANGE LEAVES[•]

Tree Condition	Inorganic Composition (Per Cent in Dry Matter)				
	Ca	Mg	K	N	P
Phosphorus-deficient	3.82	0.28	1.30	3.47	0.082
Slightly phosphorus-deficient	4.72	0.31	1.17	2.77	0.147
Ample phosphorus	4.66	0.31	1.18	2.77	0.147
High phosphorus	4.82	0.30	1.18	2.72	0.168

Source: Chapman and Rayner (1951).

[•] Average of analysis for June, July, and August leaf samples.

Soil Analysis Values.—Aldrich and Buchanan (1954) reported that the water-soluble phosphate (PO_4), as determined by the Bingham (1949) method, usually had less than 0.3 ppm of phosphate in the citrus orchard soils showing phosphorus deficiency symptoms. However, they found some orchards low in phosphorus where no signs of phosphorus lack were apparent, and, conversely, one phosphorus-deficient orchard where the water-soluble phosphate was 3.21 ppm. In Florida, Reuther *et al.* (1949) got no yield response to phosphate in a soil showing 2.8 ppm of water-soluble phosphorus.

Acid-soluble phosphorus, as determined by the Truog (1930) method, was found by Aldrich and Buchanan (1954) to be less than 30 ppm of phosphorus in orchards where phosphorus deficiency was evident. However, they found one phosphorus-deficient lemon orchard on a soil showing 226 ppm of acid-soluble phosphorus. In Florida, Forsee and Neller (1944) reported 8 pounds per acre of acid-soluble phosphorus (method of extraction not stated) in an orchard responding to phosphate. Reuther *et al.* (1949), using the method of Truog (1930), reported acid-extract values of 85 ppm of phosphorus in an orchard soil where no yield response was obtained.

As regards total phosphorus, Aldrich and Buchanan (1954) usually found less than 300 ppm of phosphorus in soils where phosphorus deficiency of citrus was present. But again there was an exception: one phosphorus-deficient orchard soil contained 1,045 ppm of total phosphorus.

From the limited data available, it is evident that soil analysis alone is not an infallible guide to citrus phosphorus adequacy. However, where tree condition and tissue analyses suggest the possibility of phosphorus deficiency, determinations of total, water-soluble, acid-soluble, and 0.5*M*-sodium bicarbonate-soluble phosphate in soils may prove useful in helping to confirm the diagnosis.

Causes of Phosphorus Deficiency.—The causes of phosphorus deficiency have been exhaustively investigated, and only a few comments will be made to indicate something about the multiple conditions under which this deficiency occurs. Low total phosphorus supply, the forms in which the phosphorus is held, presence of excess lime, competition by soil organisms for available supplies of phosphorus, too much nitrogen fertilizer (perhaps associated with high-soluble calcium), scion-rootstock combinations, climatic factors, insufficient magnesium, and soil moisture deficits are all possible factors.

Because of the variability of soils and plants, and of plant requirement and feeding power, no one of these conditions automatically implies phosphorus deficiency. The total phosphorus of soil varies widely. In some soils values as low as 0.004 per cent of phosphorus are found, and, in others, values over 0.5 per cent occasionally occur, but the majority of soils range from 0.01 to 0.18 per cent. Low total phosphorus does not necessarily mean that responses to phosphate will be obtained, nor does low water- or acid-soluble supply in itself always imply a need for supplemental phosphorus.

However, in the majority of soils requiring supplemental phosphorus, it is found that either water-soluble phosphorus is low, the supply of weak acid- or sodium bicarbonate-soluble phosphate is low, or the total supply of phosphorus is low.

It has long been known that the phosphorus supply of soils consists of both inorganic and organic forms, the latter from plant residues and microorganisms. Although extensive research on the mineralogical and inorganic chemical aspects of soil phosphorus has been made and it is usually possible to isolate and identify a few phosphate-bearing minerals from the coarser fractions of a soil (such as apatite), the actual isolation and specific identification of most of the inorganic phosphorus compounds of soil are still impossible. Therefore, most of what we know about the inorganic fraction has resulted from studies of the differential solubility of soil phosphorus in water, acid, and alkali extracts; studies of fixation by hydrous iron and aluminum compounds and by various clay minerals; and, of course, studies of the calcium chemistry of phosphorus.

It is generally agreed that in acid soils inorganic phosphate is predominantly tied up in iron and aluminum compounds of variable composition, whereas, in neutral or alkaline soils in which the prevailing constituent is calcium, most of the inorganic phosphate is in various calcium forms. In both acid and alkaline soils, some phosphorus is absorbed by the clay minerals, and some investigators believe the iron, aluminum, and calcium forms may also be tied to clay minerals.

The nutritional researches of Parker (1927), Tidmore (1930), and Parker and Pierre (1928) established that some fast-growing plants (such as corn, barley, sorghum, and tomato) can achieve maximum growth if solution concentrations are maintained at from 0.10 to 0.50 ppm of phosphate. However, it was noted by Parker (1927), Parker and Pierre (1928), and Tidmore (1930) that the displaced soil solution of some productive soils had less than these concentrations of phosphate in the soil solution, implying that root-soil contact feeding also is important in phosphorus nutrition. Working with citrus, Chapman and Rayner (1951) found that maintained concentrations of 2.5 to 3.5 ppm of phosphate in solution cultures were insufficient for most citrus needs, and yet citrus could secure adequate phosphorus from soil where quick-growing field crops failed, and where soil solution values were much lower than the 2.5 to 3.5 ppm range in the water cultures. This suggests that root-soil contact phenomena are important in the phosphorus nutrition of citrus. Additional support for this belief is found in the total and water-soluble phosphate found in the soils of citrus orchards which do and do not respond to phosphorus fertilization. The soils in which responses to phosphate occur usually show less than 0.3 ppm of phosphate in water extracts, though some exceptions have been found. In these soils, possibly special rootstock-scion interrelations or some associated soil condition limiting phosphate uptake by the plant may have intervened.

While not a great deal of specific information is available on rootstock effects in relation to phosphorus nutrition, Aldrich and Buchanan (1954), studying a soil containing 3.2 ppm of water-soluble phosphate by the Bingham method, 226 ppm of acid-soluble phosphorus, and 1,045 ppm of total phosphorus, found that lemons on rough lemon rootstock showed phosphorus deficiency symptoms, while in adjacent rows the same scion on sweet orange roots showed no phosphorus deficiency symptoms. In a phosphorus fertilized orchard in Florida, Smith *et al.* (1949) showed small, but significant effects of various rootstocks on the phosphorus concentration in leaves of Valencia oranges.

A number of field cases of phosphorus deficiency, e.g., Bouma (1959) and Embleton *et al.* (1952), have developed where soils had become acid due to the continued use of ammonium sulfate.

Phosphorus is less available in many plants where moisture stresses periodically occur. Hibbard and Nour (1959), for example, found that increasing moisture stresses decreased the leaf level of phosphorus in peach, apple, mustard, and

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strawberry. Olsen, Watanabe, and Danielson (1961), working with corn, found that phosphorus uptake by corn seedlings on a relative basis was 100, 94, 50, 50, and 35 for ⅓, ½, 1, 3, and 9 bars of soil moisture tension, respectively. Power, Reichman, and Grunes (1961), working with wheat in field trials, found similar moisture tension-phosphorus uptake relations.

From what is known about phosphorus deficiency causes generally, one would look for phosphorus deficiency of citrus on the following types of soils: (1) those which are acid and in which water or weak acid extract show low values; (2) soils which have low total supplies, i.e., less than 0.03 to 0.05 per cent of phosphorus; (3) soils which contain free lime; and (4) soils which have a long cropping history, irrespective of soil type. Modifying influences would be rootstock, climate, moisture, biological activity, and chemical factors of the soil such as soluble calcium, nitrogen, and zinc.

Control of Phosphorus Deficiency.—Although nearly all soils fix phosphorus, it has not proven difficult to correct phosphorus deficiency of citrus by broadcast soil applications of soluble phosphate fertilizers. The bulk of the feeder roots of citrus are found in the surface 18 inches of soil; in orchards where noncultivation is practiced, many feeder roots are commonly found two to four inches under the soil surface; and in cultivated orchards, many roots are found immediately below the cultivated zone. Except with soils of unusual fixing power, there is some slow migration of phosphorus downward, especially in neutral or alkaline soils. In a detailed study of phosphorus status of citrus orchards made by the author some thirty years ago (Chapman, 1934), it was found that substantial movement of phosphorus had taken place into the second 6 inches of most orchard soils and, in most, some movement into the second and even third foot. These were in orchards which had had repeated applications of manures and/or other phosphate-carrying fertilizers. Organic matter seemed to aid in the rate of phosphorus movement, as heavily manured soils showed deeper penetration than those not receiving organic matter.

Pratt, Jones, and Chapman (1956), studying phosphorus distribution in an irrigated orchard soil after twenty-eight years of using superphosphate and/or manure, found 80 per cent of the applied phosphate in the top 12 inches of soil, but there was evidence of some movement beyond the 24-inch depth. They also noted a buildup under the trees due to leaf drop. There is usually root development in the area beneath the trees, even though under irrigation agriculture it is drier than the irrigated areas. However, there is always some lateral movement of moisture from furrows at the drip of the tree, and, when rains occur, good moisture conditions then prevail under the tree.

Because of the known fixing power and slow rate of phosphorus movement in soils, Aldrich and Coony (1952), in seeking to quickly correct phosphorus deficiency, applied 20 pounds of ammonium phosphate (11–48) per tree, broadcast around the dripline (skirt) of the trees. (This amounted to 9.6 pounds of P_2O_5 per tree.) Within four months marked responses to the phosphate occurred. The soils of these experiments were moderately acid sandy loam and loamy sand types. Leaf analyses made six months to a year following treatment showed increased phosphorus absorption by the tree. Embleton *et al.* (1952) found that phosphoric acid (75 per cent), applied at rates of 16 pounds per tree per year, corrected phosphorus deficiency, as did treble superphosphate. The probabilities are that lower amounts of phosphate would suffice, probably 5 pounds of P_2O_5 per tree or less. Fuller and Hilgeman (1955) applied radioactive phosphoric acid at rates of 5 pounds of P_2O_5 per tree to a circular basin 6 feet in diameter around navel orange trees and noted absorption of 19 to 24 per cent of the radioactive phosphate in a few months. The soil was a calcareous clay loam. There was no significant difference in absorption rate by rough lemon and sour orange rootstocks.

Nakama *et al.* (1962) applied 79.7 gm of superphosphate tagged with 470 μc of P^{32} to a nineteen-year-old satsuma tree growing on a terraced gravelly sandy soil. The application was in July, and by November 20, 0.5 per cent of the P^{32} applied had been absorbed by the tree. Some 27 per cent of the P^{32} was retained in the upper 9 cm of soil. There had been 1,085 mm of rain during this period. The rest of the phosphorus must have migrated to lower soil depths, but the P^{32} was so diluted as not to be detectable.

It is also possible to secure some correction of phosphorus deficiency by sprays containing soluble phosphorus (Sato, Ishihara, and Harada, 1954). But the correction is temporary, necessitating repeated sprayings, and spray residue encourages insect buildup and involves additional machine traffic in orchards. Therefore, except in

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unusual circumstances, reliance on phosphate sprays to correct deficiency of this element is not recommended.

As to the form of phosphorus best suited for soil applications, soluble forms such as treble superphosphate or ammonium phosphate are recommended. From special penetration trials, it appears that the former may move a little more readily than the latter (Chapman, unpublished data). Applications of P_2O_5 at the rate of 5 or more pounds per tree around the drip of the tree will usually result in correction for several years.

In calcareous soils where phosphorus deficiency is due to the low solubility of phosphorus, Chapman (1936) found that the use of soil-acidifying agents, such as sulfur and ammonium sulfate, would increase the availability of phosphorus, provided these materials were present in the area where absorbing roots are present.

DeRemer and Smith (1961) have suggested from work with various chelates in sand cultures that these compounds can react with insoluble iron and aluminum phosphates to release soluble phosphate. In acid soils, when phosphorus is tied up in iron and aluminum forms and not readily available, chelates might prove very useful.

Excessive applications of phosphate should be avoided because of the effects on the availability of other nutrients.

Phosphorus Excess

Unlike potassium, boron, and some other elements, phosphorus is not excessively absorbed to the point of producing direct injury symptoms. It does accumulate in the plant to some degree under conditions of nitrogen and zinc deficiencies. In early work on inorganic phosphate in citrus, Chapman (1935) found especially high concentrations of PO_4 in both leaf and woody tissues of zinc-deficient trees.

The chief ill effects of high phosphorus are on the availability and/or absorption or utilization of other elements. There is now abundant evidence that phosphate in soils can decrease the availability of copper, zinc, boron, iron, and other micronutrients and may affect nitrogen nutrition unfavorably.

With respect to nitrogen, Chapman and Brown (1941*a*), in a fertilizer experiment with orange trees in 55-gallon oil drums, found that heavy phosphate applications produced nitrogen-starvation symptoms. Lilleland (1933) noted similar effects on deciduous fruit trees. Anderssen (1937) and Anderssen and Bathurst (1938) noted reciprocal relations between nitrogen and phosphorus, high nitrogen trees having low phosphorus and low nitrogen trees having high phosphorus. There is much evidence to support the view that the ill effects of too much nitrogen, as it affects fruit quality, can be offset by increased phosphorus. In field experiments, Heymann-Herschberg (1952) found less nitrogen in leaves where phosphorus had been applied.

With respect to micronutrients, Chapman, Vanselow, and Liebig (1937) found that in controlled cultures zinc deficiency was accentuated by high phosphorus, and West (1938) found in field trials involving superphosphate that zinc deficiency was worse in plots receiving this fertilizer than in plots not getting phosphate. Results with many crops, both in the field and in pots or controlled cultures, are in harmony with these findings and have been noted by others: Bathurst (1945); Reuther and Crawford (1946); Mowry and Camp (1934); Chapman *et al.* (1940); Thorne and Wann (1950); Labanauskas, Embleton, and Jones (1958*a*); Loneragan (1951); Millikan (1947*a*); Rogers and Wu (1948); Bingham and Martin (1956); Bingham *et al.* (1958); Burleson, Dacus, and Gerard (1961); and Langin *et al.* (1962). However, some workers have found no significant interaction: Boawn, Viets, and Crawford (1954); Thorne (1957); Seatz, Sterges, and Kramer (1959); Bingham (1963) and Smith, Scudder, and Hrnciar (1963). Some believe that the phosphate effect on zinc is due more to reaction or effects within the plant or plant roots than in the soil. Boawn and Leggett (1964), working with potatoes, found that the phosphorus-zinc ratios in the plant determined whether or not the plant was zinc-deficient. Healthy plants had phosphorus-zinc concentration ratios of < 400, and zinc-deficient plants had ratios of > 400. It seems likely that this same effect may take place in the soil, and that zinc uptake from the soil as well as utilization within the plant depends upon phosphorus-zinc ratios in which not only zinc solubility, but competition for absorption sites on the root and carriers (chelates) in the plant and associated cation-anion relations are involved.

Even more striking effects of phosphate on copper availability have been noted. Forsee and Neller (1944) and Forsee and Allison (1944) found on an acid peat that phosphate increased the amount of ammoniation (copper deficiency).

Reuther *et al.* (1949) found that phosphate fertilization decreased the copper content of citrus leaves, but increased the zinc and manganese contents. In a series of experiments with soil in pots, Bingham and Martin (1956), Bingham *et al.* (1958), Bingham and Garber (1960), and Bingham (1963) found with many soils and several forms of phosphate that copper intake by citrus was markedly reduced by increased phosphate addition.

In recent work with four different soils in pot cultures, Martin and Van Gundy (1963) observed that additions of calcium phosphate markedly decreased the copper content of the leaves.

With respect to other micronutrients, Bingham and Garber (1960) reported that high applications of phosphate reduced boron intake, but increased manganese absorption. Reuther *et al.* (1949) also determined that leaf manganese was increased by phosphate, and Chapman and Brown (1941*a*) found that manganese deficiency developed when phosphorus was deficient and disappeared when phosphate was added to soils.

In nutrient cultures of sweet orange seedlings, Chapman *et al.* (1940) found that iron chlorosis was produced by high phosphorus concentrations. Iron-phosphate interactions have been noted by others, e.g., Olsen (1935).

Phosphate applications commonly increase molybdenum absorption (Anderson, 1956).

Creasing of Fruit.—There is some evidence that excessive phosphorus or unbalanced nutritional conditions (especially low potassium and nitrogen) may lead to creasing, or "crinkle rind," as it is sometimes referred to.

In the Citrusdal region of South Africa, Fourie and Joubert (1957) and Cooper (1958) reported that phosphorus fertilization seemed to increase the amount of this trouble, especially if potassium was low. On the other hand, the latter element decreased creasing, though in all cases the amount of this disorder varied from year to year, indicating a strong climatic influence.

Embleton *et al.* (1952) noted that an increase in creasing occurred on the plots of a phosphorus-deficient soil receiving phosphate.

Haas (1950) found that high phosphorus in his cultures increased the amount of creasing.

Anderssen (1937) noted that greater waste of fruit in storage occurred on the low nitrogen (high phosphorus) fruit. In their study of variable phosphate supply on navel oranges under outdoor water culture, Chapman and Rayner (1951) noted in one of the years of their experiment a greater amount of creasing in some of the trees of the high phosphate group. It is apparent that size of crop, climatic factors, and nitrogen-phosphorus-potassium nutritional relations are all involved in the creasing problem.

Biological Effects.—In outdoor water-culture experiments with citrus, Chapman and Brown (1942) found that high phosphate encouraged a marked root infection with *Thielaviopsis basicola* fungus; very little infection was found in the low phosphate cultures. By decreasing the acidity of the nutrient cultures to pH 4, it was possible to suppress the growth of this fungus even in the presence of high phosphate.

In a study with the citrus nematode *(Tylenchulus semipenetrans)*, Martin and Van Gundy (1963) found that high phosphate additions decreased the nematode population and that sweet orange seedlings, though depressed in growth by the nematodes, were less depressed in growth by the high phosphate than by low phosphate. Phosphate rates were at 0, 76, 360, 900, and 1,800 pounds of phosphorus per acre. The reduction in nematodes was quite marked at the two latter rates on three of the four soils used.

From this brief review, it is evident that tree nutrition can be profoundly influenced by excess phosphorus, acting mostly through indirect effects on the chemistry and biology of the soil.

POTASSIUM (K)

Potassium Deficiency

Most of the detailed information about the effects of potassium deficiency and excess on citrus has come from controlled experiments in sand and solution cultures. Results obtained under these conditions harmonize well with various descriptions which have emerged from field experiments. Unlike many crops where the effects of potassium deficiency produce specific and easily recognizable symptoms (such as marginal and interveinal leaf scorch on apple and peripheral spotting on alfalfa and clovers), those on citrus are so varied and multiple that no one symptom taken by itself is sufficient to positively identify it as due to lack of potassium. (See table 3–30 for a summary of foliage and fruit effects.)

Reed and Haas (1923*a*) grew young orange trees in sand culture without potassium for seventeen months. Only the earliest stages of potassium deficiency were produced. Leaf size was reduced; there was some loss of chlorophyll in the leaves; and some iron chlorosis was seen. Later, Haas

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(1936*c*) grew young citrus trees in sand cultures lacking potassium and noted some necrosis on leaves, twig dieback, and gum exudation on wood. Some of the leaves showed resinous spots. Bryan (1935) grew grapefruit seedlings in potassium-deficient cultures and noted retardation of growth, leaf puckering, and a fading of chlorophyll in small, irregular spots on the leaves. Pustular brown spots also appeared in the leaves. The branches drooped and showed a lack of rigidity. Eckstein,

Table 3–30

PRINCIPAL POTASSIUM DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Potassium Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Retardation of growth and leaf size is first effect.	1. Many other nutritional disorders, as well as drought, insects, and diseases can produce similar effects.
2. Twisting, curling, puckering, and cupping of leaves come on as deficiency becomes moderate to acute.	2. Aphid infestations on new growth produce a marked deformation of leaves; injuries to young leaves and other factors can cause some twisting.
3. Excess leaf drop in the spring following bloom; many of these leaves are very yellow, more so than the usual yellowishness of senescent leaves. This effect was not seen on grapefruit in Florida (Smith and Rasmussen, 1961).	3. Boron and salt injury and other deficiencies can cause excessive leaf drop.
4. Dieback of weakened twigs.	4. Any condition which injures leaves and weakens growth can bring on dieback.
5. Production of weak, small, new shoots, some of them showing yellow color at the point of attachment to twigs. Bark may show sunken or pitted areas in yellowed region. There is a tendency for these yellowish weak shoots to abscise easily, also to drop and produce S-shapes.	5. Boron and other deficiencies can cause weak, easily aborted new growth, but not the yellow color and pitted or sunken areas in young stem tissue.
6. Foliage loses chlorophyll and looks bronzed or yellowish in a nonuniform way. This effect increases with severity of deficiency.	6. Phosphorus and nitrogen deficiencies can produce much this same appearance.
7. Some of the leaves may show speckling of brown spots; portions of midribs and veins may turn yellow. Some necrotic areas and spotting may develop.	7. Some of these same conditions can arise from other causes.
8. There may be some iron chlorosis.	8. Iron deficiency; excess zinc, and excess soil moisture.
9. There may be more sunburning of leaves on the sunny side of the tree.	9. Nitrogen and phosphorus deficiencies also may cause these effects.
10. Gum exudation on limbs and trunk may occur.	10. Boron deficiency, red scale infestation, and copper deficiency can cause gum exudation.
11. Bloom may decrease slightly in early deficiency stages, but not enough to decrease fruit set. In more advanced stages, bloom is considerably decreased.	11. Phosphorus and nitrogen deficiencies.
Fruiting and Fruit Characteristics	
1. Fruit on trees slightly, moderately, or acutely deficient is smaller and turns color sooner than fruit from trees getting ample potassium.	1. Lack of magnesium, zinc, iron, and other elements can decrease fruit sizes. Also heavy bearing and moisture stress.
2. Rind texture smooth and peel thin.	2. Lack of nitrogen; heavy yields.
3. Juice percentage good and internal quality not especially impaired.	
4. Compared to fruit getting ample potassium, acid may be a little less, but total solids may not be much affected or may be slightly higher.	
5. Fruit splitting has been found by Koo (1963) to be greater where potassium is low.	5. Copper deficiency; other conditions (weather) probably are involved.
6. Creasing may be greater where potassium is low.	6. High phosphorus and weather conditions.
7. Yield is not decreased much, if any, in very early stages of deficiency, but later fruit drop may be marked.	
8. Keeping quality in storage is decreased by lack of potassium.	

Tissue Analysis Values

1. Mature leaves from acutely deficient trees may range from 0.06 to 0.25 per cent of potassium in dry matter.
2. Fruit sizes (other factors, such as nutrition, moisture, heat units, etc., being favorable) may be increased if potassium in leaves is in the 0.3 to 1 per cent range and uptake of potassium is increased to 1.25 to 1.75 per cent levels.
3. Calcium, magnesium, sodium, boron, and nitrogen may increase in leaves from potassium-deficient trees; iron may decrease.
4. For best performance, leaf levels of from 1.20 to 1.70 per cent of potassium in dry matter are recommended, unless early fruit maturity is desired or smaller sizes of fruit are required.

Bruno, and Turrentine (1937) cite leaf puckering along the midribs and brown necrotic spots on leaves as indicative of potassium deficiency.

Oppenheimer and Mendel (1938) grew sweet lime cuttings in sand cultures receiving variable potassium supplies and noted that where potassium was lacking there were brown spots on the tips and margins of leaves, a yellow coloration followed by necrosis of the youngest leaves and buds, premature defoliation, and bronze discoloration of branches. The potassium levels of leaves from the plants of deficient cultures ranged from 0.42 to 0.79 per cent of potassium in the dry matter, compared with values of 1.65 to 4.14 per cent in normal plants.

Chapman and Brown (1943) grew sweet

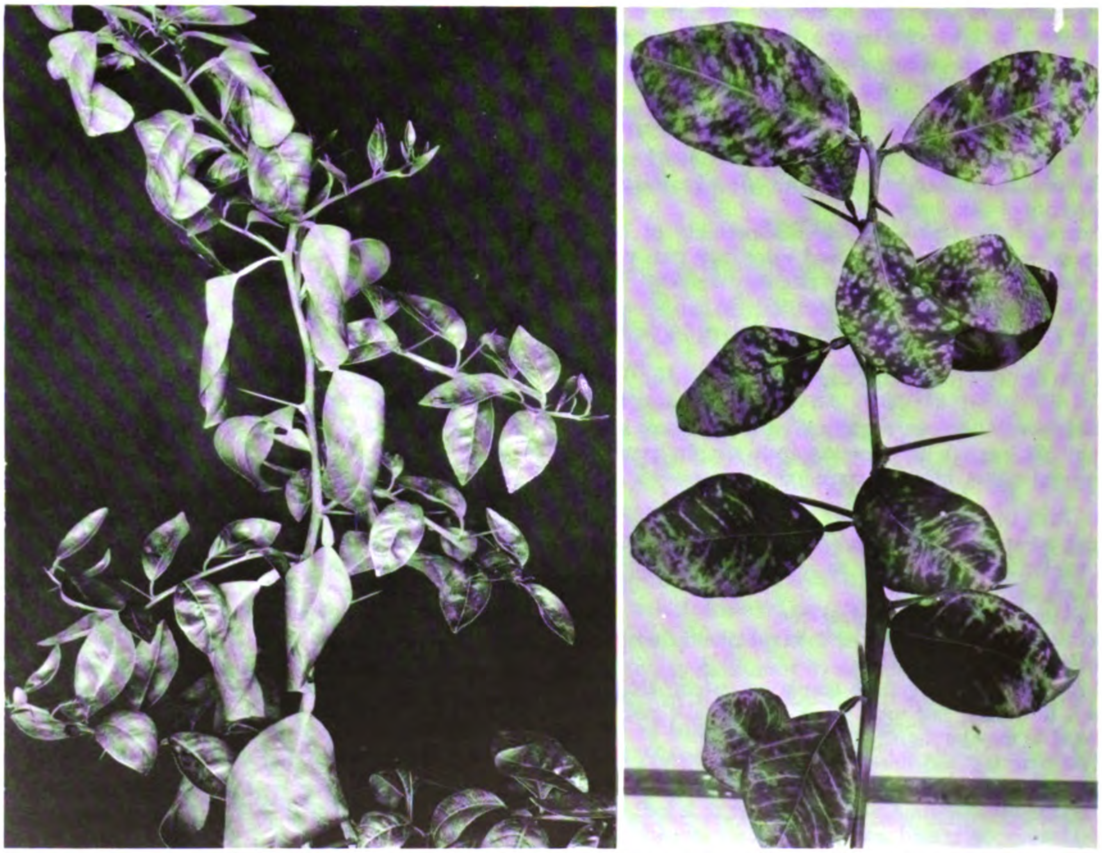

Fig. 3–47. Effects of potassium deficiency. Left, leaf crinkling and curling and weak, multiple shoots on sweet orange seedling. Right, extreme case of leaf spotting and partial vein chlorosis on grapefruit seedling.

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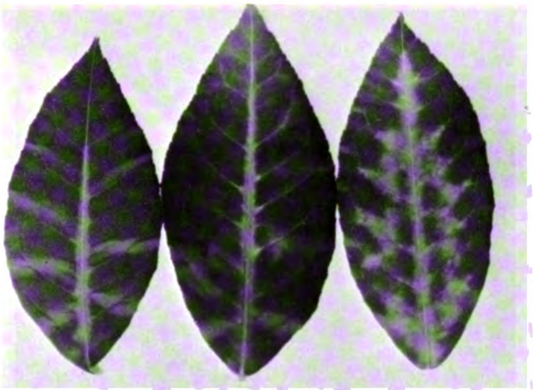

Fig. 3–48. **Potassium-deficient lemon leaves showing various types of vein clearing and yellowing.**

orange, grapefruit seedlings, and lemon cuttings in sand cultures for twelve months under potash-deficient and nondeficient conditions, varying the nutrition of the two sets of cultures so as to produce potassium deficiency under widely variable nutrition as regards nitrate, sulfate, phosphate, sodium, calcium, and magnesium. Though the onset of potassium deficiency was delayed by high sodium and hastened by high calcium and magnesium, the same fundamental growth and leaf characteristics ultimately developed in all cultures. Reduced growth rate, weak lateral shoots, decreased leaf size, leaf puckering and curling, with a series of patterns and markings characterized by some vein clearing, yellow spots or stippling, resinous spots, and some necrosis were among the main effects noted. Some of these symptoms are illustrated in figures 3–47 through 3–51.

Some of the weak new shoots showed sunken or pitted areas in the bark and wood and abscised prematurely (fig. 3–52). There were no irregular marginal and interveinal leaf-scorch patterns and effects, as noted for so many other plants. Leaf burn from excess sodium accumulation in the high-sodium cultures was more pronounced where potassium was deficient. Also, boron tended to accumulate more in the leaves of potassium-deficient plants than in those receiving ample potassium. Depriving the potassium-deficient plants of water by allowing them to wilt did not bring on any additional symptoms such as leaf scorch. There was some transient iron chlorosis in most of the cultures.

Analyses of leaves from the potassium-deficient cultures showed total potassium in the dry matter ranging from 0.06 to 0.25 per cent, and calcium, magnesium, and nitrogen were always higher in these than in the controls.

To gain a more complete understanding of the growth and visual symptoms produced on bearing trees growing outdoors, as well as effects on fruit yield and quality, four-year-old navel and Valencia orange trees on sour orange rootstock

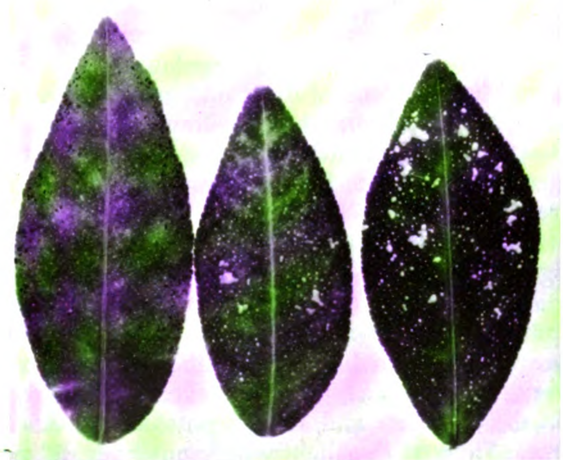

Fig. 3–49. **Potassium-deficient lemon leaves showing speckling, brown resinous spots, some vein clearing, and necrotic spotting.**

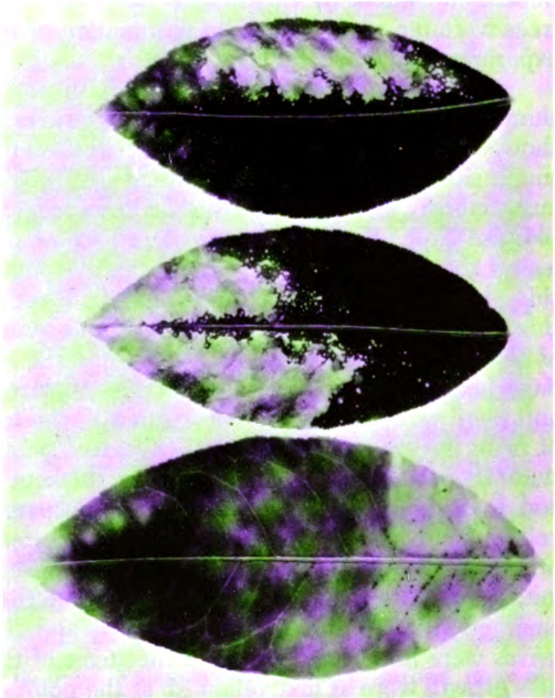

Fig. 3–50. **Potassium-deficient lemon leaves showing some apex mottlings (left), due to boron accumulation, leaf burning, and uneven fading.**

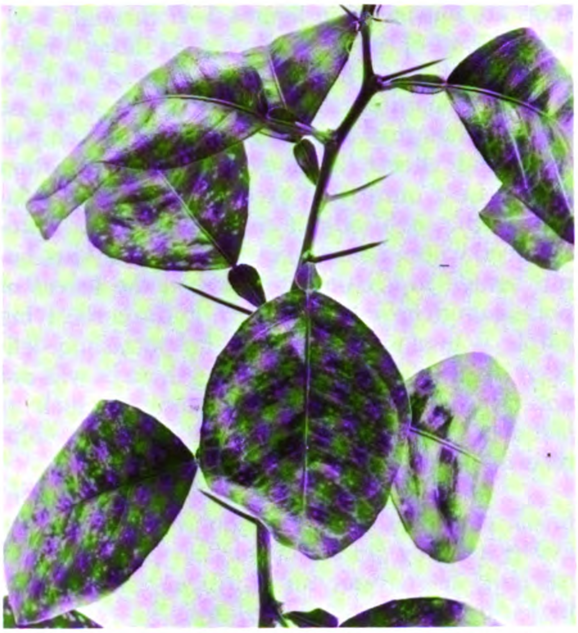

Fig. 3–51. Potassium-deficient grapefruit shoot showing curling of leaves, vein clearing, and chlorotic spots.

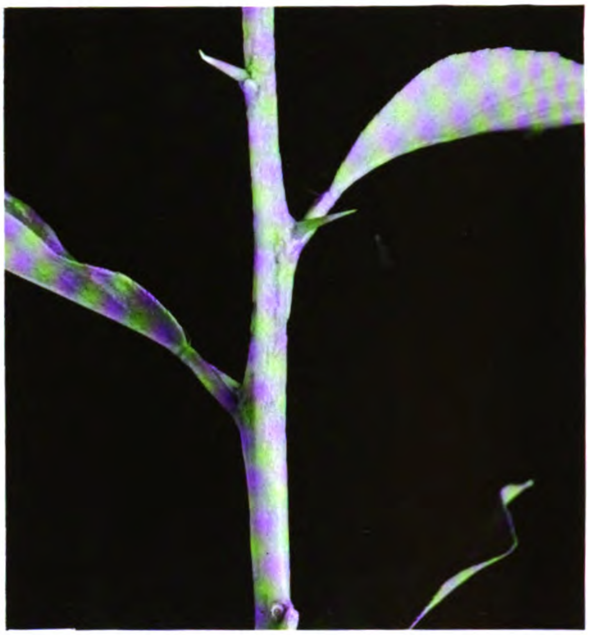

Fig. 3–52. Enlarged section of a young succulent lemon stem showing sunken or pitted areas in the bark.

were grown for six years in solutions of various potassium content by Chapman *et al.* (1947). The trees were ten years old at the termination of the experiment.

Trees originally well supplied with potassium when deprived of this element gradually showed less new growth; some of the leaves on the sunny side showed a greater tendency to bronze and sunburn, and curled a little. In the spring at blossom time, there was a greater drop of old leaves than on the trees receiving ample potassium, but the amount of blossoms and fruit set at this stage were not seriously affected. As a result of less new growth and greater leaf drop, the foliage became thinner, dieback of twigs occurred, and there was pronounced curling and puckering of many leaves during the second year of potassium starvation. Such leaves became thickened, and many of them became yellowish or partly yellow, but with no one fixed or particular pattern predominating as in iron, zinc, manganese, and magnesium deficiencies. The new growth that emerged from time to time was weak, and the leaves were undersized. Some of the stems of the new growth were at first yellowish at the point of attachment to the longer twigs, and because of stem weakening, there was a tendency to sag and develop an S-curvature.

In subsequent years, as the deficiency became very acute, dieback, leaf drop, sparse bloom, and yellowed, crinkled foliage became very pronounced. There was also some iron chlorosis on some leaves. If iron sulfate solution was painted on the leaves, green spots appeared, as occurs with typical iron chlorosis.

Trees held at potassium levels producing only a very slight deficiency showed nothing in the way of foliage color, density, or leaf size symptoms, as contrasted to trees receiving ample potassium. However, in the spring there was a somewhat greater leaf drop, and many of the abscising leaves were more yellowish than those that normally characterized the leaf fall at this time of year from control trees. Photographs of trees at the very earliest stage and at the acute stage of potassium deficiency are presented as figures 3–53 and 3–54.

The chief distinguishing feature on the trees only slightly lacking in potassium was the production of smaller-sized fruit. This feature was very consistent and, of course, even more marked on the moderate and acutely potassium-deficient trees. Yield on the slightly deficient trees did not appear to be affected. The fruit on these and the more potassium-deficient trees colored earlier than those on trees receiving ample potassium.

Table 3–31

EFFECT OF VARYING POTASSIUM ON NUMBER AND WEIGHT OF FRUITS

		1943–44		1944–45		1945–46		Total and Average 1943–46	
Potassium	Number of Trees	Number of Fruit Picked	Average Weight per Fruit (Grams)	Number of Fruit Picked	Average Weight per Fruit (Grams)	Number of Fruit Picked	Average Weight per Fruit (Grams)	Number of Fruit Picked	Average Weight per Fruit (Grams)
Severe deficiency	4	31	115	0	0	101	108	132	109
Slight deficiency	5	562	137	163	136	741	119	1,466	128
Ample potassium	5	231	263	407	144	525	150	1,163	170

The effects of varying potassium supplies on fruit size, as found in this six-year experiment, are shown in figures 3–55 and 3–56. Data taken from Chapman *et al.* (1947), relating potassium status of trees to fruit size, are given in table 3–31.

The fruit produced on trees slightly and extremely deficient in potassium was smooth in rind texture, thin-skinned, juicy, and of good acid and total solids content (though generally lower in acid than the high potash-treatment fruit). Excess potassium made for large fruit, coarse rinds, thick skins, coarse flesh, and poor eating quality. Fruits and twigs from potassium-deficient and nondeficient orange trees in this experiment are shown in figure 3–57.

The size-reducing effect of potassium lack or the size-increasing effect of ample potassium has been noted in field trials by Benton and Stokes (1925) and Benton and Stokes (1931), in Australia; Bahrt and Roy (1940), Neetles and Forsee (1941), and Reuther and Smith (1952), in Florida; Parker and Jones (1950), in California; Winnik (1950), in Israel; Deszyck and Koo (1957), in Florida on grapefruit; on Hamlin and Valencia oranges in Florida by Deszyck, Koo, and Ting (1958); and on Ponkan mandarin in Taiwan by Chu (1963). Samson (1960) got increased sizes of oranges after applying potash in a fertilizer experiment in Surinam.

In pot cultures of soil using lemons (on sour orange rootstock), Haas and Brusca (1955*b*) found that periodic additions of potassium increased lemon fruit sizes and the potassium content of the flowers, peel, and pulp. In pot cultures in Japan, Ishihara, Hase, and Sato (1965) found increased potassium fertilization increased yield, fruit size, and keeping quality of satsuma in storage when nitrogen fertilization was adequate. At a low nitrogen level, heavy potassium reduced yield and fruit size.

In a sand-culture trial in Florida, Smith and Rasmussen (1959) found that adding potassium to young Valencia orange trees (on rough lemon rootstock) increased fruit sizes and rind coarseness. Reciprocation with calcium or magnesium made no difference in the potassium response.

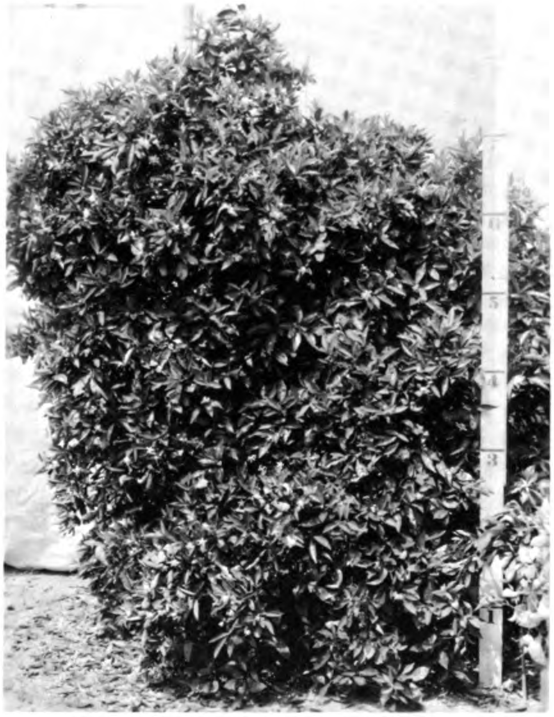

Fig. 3–53. Valencia orange tree in earliest stage of potassium deficiency. Heavier-than-normal leaf drop at bloom period, small-sized fruit, and low potassium in the leaves are the chief distinguishing effects at this stage.

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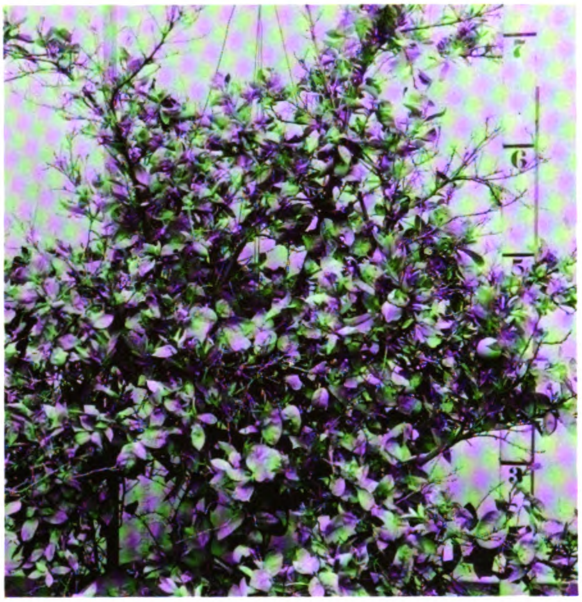

Fig. 3–54. Valencia orange tree showing effects of acute potassium deficiency. Note dieback, curled leaves, and defoliation.

Embleton and Jones (1956) have reported on field experiments in which potash application reduced orange fruit sizes. Magnesium uptake was reduced by the potassium, and it is known that lack of this latter element will result in smaller fruit sizes.

Many other conditions can reduce fruit sizes, such as periodic water deficits, lack of sufficient heat units, desiccating winds, lack of other nutrients, as well as virus diseases, nematodes, root-attacking fungi, and insect infestations. Hence, it cannot be expected that increased potassium supplies will necessarily improve fruit size under all circumstances.

However, barring the foregoing complications, the evidence is clear that low potassium (before growth or other visual symptoms appear) will reduce fruit size. Conversely, even though the total number of fruits borne may not increase, increasing potassium beyond sufficiency levels will increase fruit size. Excess conditions decrease quality by producing fruits of coarse, thick rinds and poor internal character and quality.

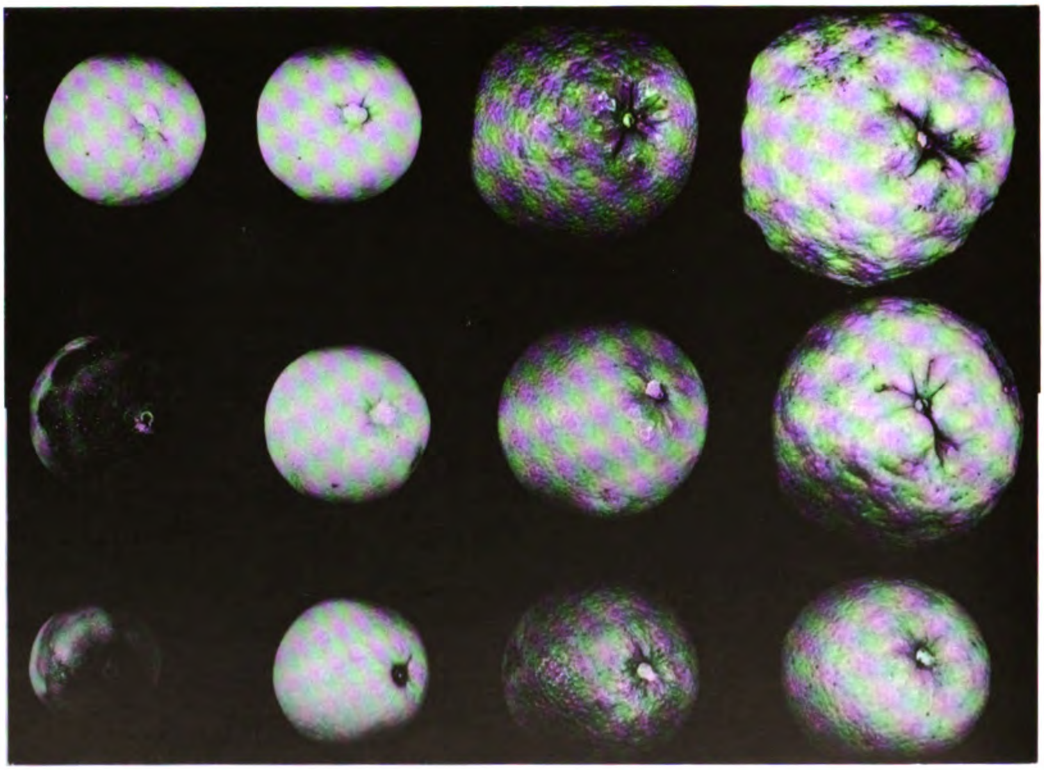

Fig. 3–55. Valencia oranges showing effects of potassium deficiency and excess. The first two vertical rows at the left show fruits from extremely deficient and slightly deficient trees; the fruits in the third row from the left are from a tree getting ample potassium; and the row at the right shows fruits from a tree getting excessive potassium.

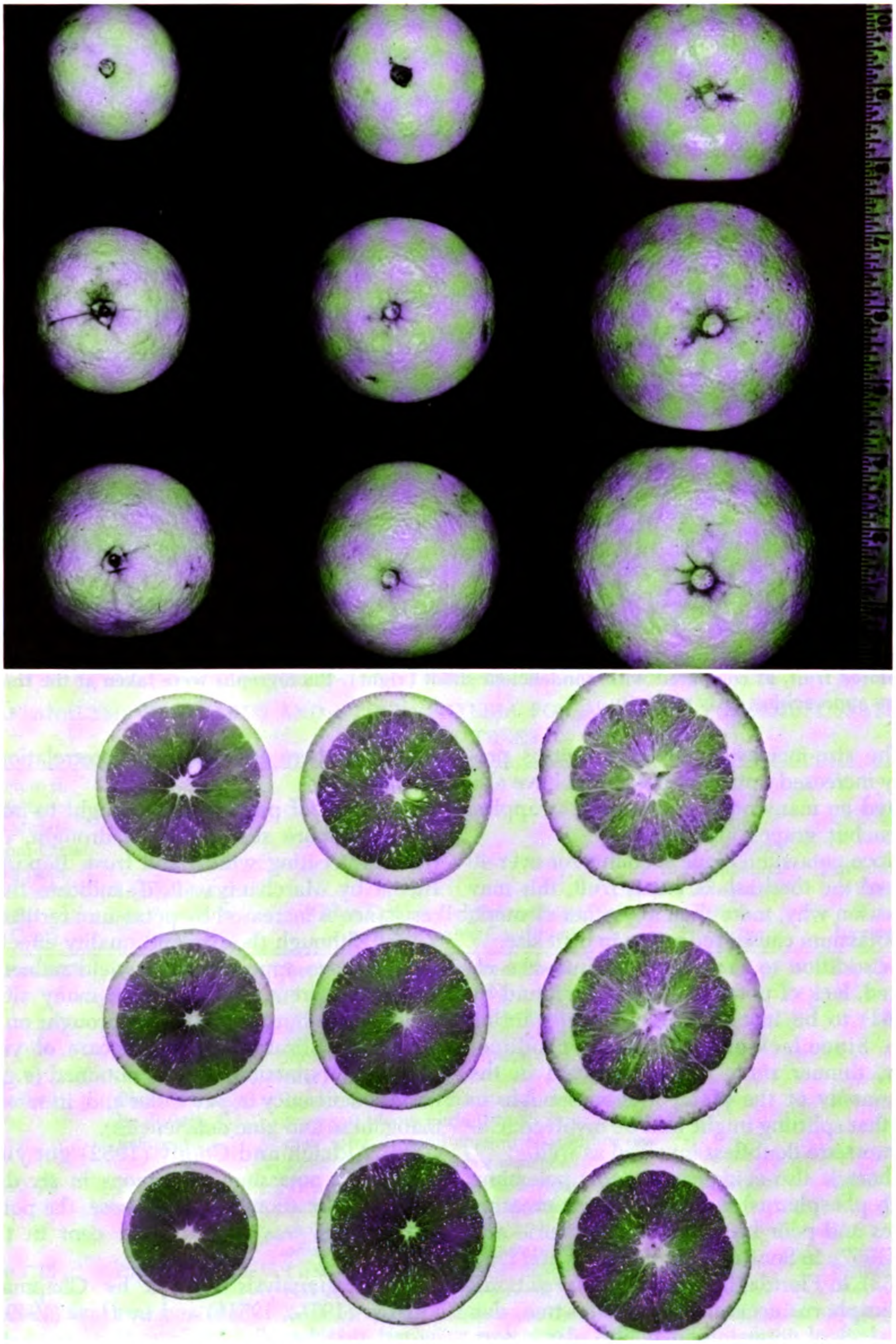

Fig. 3–56. Navel oranges showing potassium deficiency and slight excess effects: very deficient (left), slightly deficient (center), and slightly excess (right).

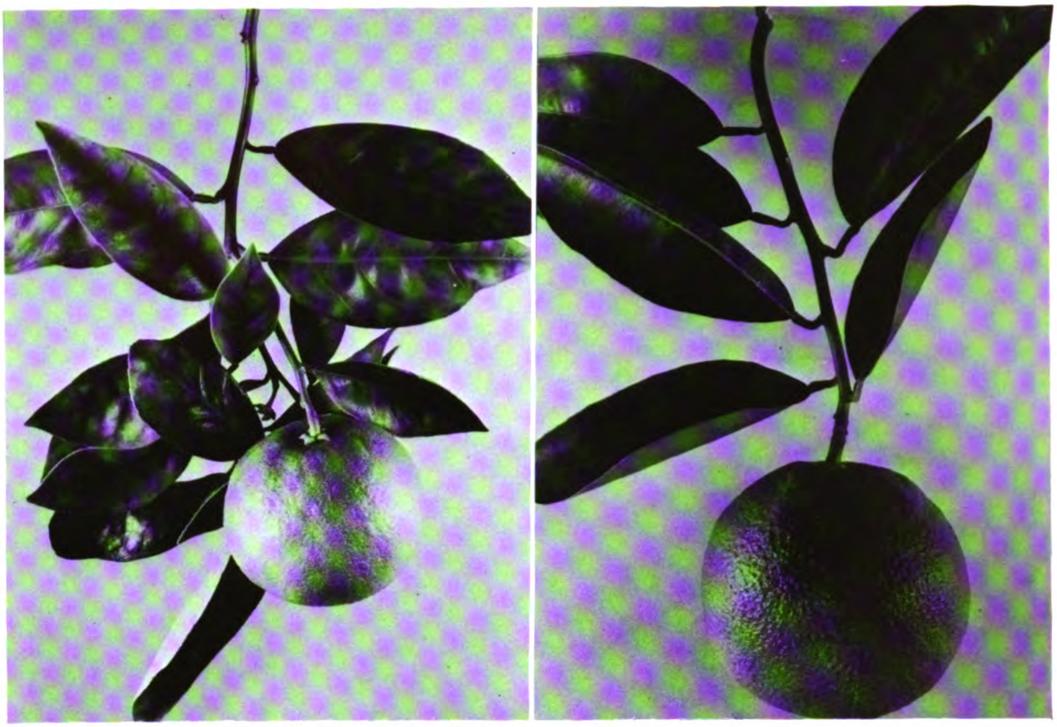

Fig. 3–57. Orange shoot from potassium-deficient tree (left), showing crinkling of leaves and smaller-sized, lighter-colored fruit, as compared with nondeficient shoot (right). Photographs were taken at the time when fruits were approaching early maturity.

The size-increasing effects on citrus produced by increased uptake of potassium have also been noted on many other fruits, such as apples, pears, peaches, grapes, and berries.

Since potassium alone accounts for over 40 per cent of the total ash of citrus fruit, this may be one reason why, more than any other element, lack of potassium causes reduction in fruit size.

In addition to the decreased fruit-size effects noted, lack of potassium has been found by Koo (1961) to be involved in the fruit-splitting problem. Since lack of this element produces smoother, thinner rinds and is involved in the water economy of the plant, it is reasonable to suppose that splitting might well be involved. Climatic factors are doubtless involved as well.

There is also evidence that low potassium (and high phosphorus) may account for creasing in oranges and poor keeping quality. Fourie and Joubert (1957), in South Africa, and Sites and Deszyck (1953), in Florida, have noted such relations.

Phosphorus accumulation in the tree, due in part to lack of potassium, may also play a part. Creasing is much more prevalent in some years than others and, like the splitting problem, suggests that there are important interrelations with climate.

Lack of potassium is thought to make citrus trees more susceptible to drought, damage from desiccating winds, and frost. Reports from Russia by Marchaniya (1957) indicate that cold resistance is increased by potassium fertilization.

Although the size and quality effects mentioned above appear prior to yield reductions, it is now apparent that there are many situations where if the nutrient disorders brought on by potassium fertilization are taken care of yield responses to potassium will be obtained (e.g., magnesium deficiency in particular and, in some cases, manganese and zinc deficiencies).

Aldrich and Coony (1952) got yield responses to potassium on lemons in six different California locations. In all of these, the potassium leaf content was below 1 per cent in the dry matter.

Leaf-analysis surveys by Chapman and Fullmer (1951*a*, 1951*b*) and by Haas (1949*b*) suggested that beneficial effects from potassium on both yields and fruit size may occur in many California citrus orchards. Subsequent field trials by

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Embleton, Jones and Page (1966) have confirmed these indications.

In an experiment with grapefruit in Florida, Sites (1951) reported yield responses from potash fertilizer and found that fruit drop was decreased and that trees were more resistant to stormy winds. Sites (1951) and Deszyck and Koo (1957), reporting on fruit quality in their experiments, found that potassium increased soluble solids, acid, and vitamin C. Fruit sizes and rind coarseness were increased by potassium. In other experiments with Valencia and Hamlin oranges, Sites and Deszyck (1953) and Deszyck *et al.* (1958) reported yield responses to potassium.

In the latter years of a field experiment at Nelspruit, South Africa, Naude (1954) obtained yield responses with Valencia oranges from the application of potash fertilizer. Fruit drop in autumn was quite marked in plots not receiving potassium.

In Australia, Arnot (1946, 1947) reported potassium deficiency symptoms of citrus on a low-lying, gray, sandy soil. Reduced yields, weak new growth, pale small leaves, dieback, pitting and necrosis of bark on young shoots, defoliation, and a low potassium content of foliage were the chief effects. In the Sydney area of Australia, Levitt (1947) reported yield increases in young citrus trees from the application of 5 pounds of potassium sulfate per tree.

Bahrt and Roy (1940) reported yield increases from potassium on a Florida soil.

Uexküll and Kämpfer (1963), reporting on a fertilizer experiment in Surinam (Dutch Guiana) by Samson (1960), indicated that substantial yield increases were obtained from both potassium and magnesium applications to a sandy soil of the coastal plain. In addition, fruit sizes and keeping quality in storage were increased by potassium.

In Taiwan, Chiu, Lian, and Hsu (1960) reported yield increases from potassium on three soil types—a yellowish-brown, clay loam, an alluvial, silty loam, and a latosol clay loam. These experiments were on trees nine to twenty years old, and they received potash (K_2O) at rates of 500 to 600 gm per tree. A similar yield response was noted in Taiwan by Chu (1963) with six-year-old Ponkan trees in the field.

Table 3–32

LEAF ANALYSIS STANDARDS AND OTHER CRITERIA FOR ASSESSING POTASSIUM STATUS OF ORANGE TREES

Potassium in Dry Matter of 3 to 7-Month-Old Bloom-Cycle Leaves from Fruit-Bearing Twigs (Per Cent)	Indications as to Potassium Status of Tree	Other Criteria Helpful in Diagnosis, and Associated Conditions	Probability of Yield Response to Application of Adequate Potash Fertilizer	Probability of Fruit-Size Increase
Less than 0.25	Potassium likely deficient	Look for crinkled, puckered leaves, more-or-less yellowing of foliage, excessive leaf shedding at bloom period, small fruit and dieback of twigs, and excessive sunburn on south side of tree following hot weather.	Good	Excellent
0.25 to 0.40	Potassium may be slightly deficient	Look for heavier than usual shedding of leaves at bloom period, small fruit; leaves may show unusual amount of sunburn on south side following hot weather.	Fair	Excellent
0.40 to 1.00	Potassium probably ample	This is the range of potash values found in healthy high-yielding orchards; if orchard is not productive, look for causes other than potash deficiency or excess.	Doubtful	Good
1.00 to 1.75	Potassium ample	Look for possible other deficiencies or conditions limiting tree growth	None	Doubtful
More than 2.50	Potassium may be excessive	Look for excess potash in soil and other conditions, including malnutrition, limiting growth of tree.	None	Fruit quality may be impaired

Source: Chapman and Brown (1950).

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Leaf Analysis Values.—The first comprehensive study of citrus leaf analysis as a diagnostic tool with special reference to potassium was made by Chapman and Brown (1950). On the basis of controlled cultures, field experiments, and a survey of the literature, the data of table 3–32 were prepared. These data, based on the analysis of three- to seven-month-old bloom-cycle leaves from fruit-bearing branches, require little or no modification today. But it is emphasized that if similar-aged leaves (bloom-cycle leaves) are taken from *nonfruiting* terminals, then the following values for per cent potassium in the dry matter of Valencia orange leaves would be more definitive. These are data from Reuther *et al.* (1958):

Condition	*Percentage of Potassium in Dry Matter*
Deficient	< 0.6
Low	0.7 to 1.10
Optimum	1.2 to 1.70
High	1.8 to 2.30
Excess	> 2.40

A more recent tabulation by Embleton, Jones, and Platt (1967) is shown in figure 3–58. This summarizes in a general way the combined experience and experimental results of many who have been working on potassium nutrition. The direction of changes are for the most part valid, but one needs to avoid a too close or literal interpretation of leaf analysis values at which changes in yield, size, quality, or other characters may occur.

Deficiencies of nitrogen, phosphorus, calcium, magnesium, and zinc are known to cause substantial accumulations of potassium in leaves. In determining potassium status and the need for potassium fertilization, leaf analyses for these and other elements need to be made so that the nutritional balance and status can be correctly evaluated.

Koo (1963) suggested that on July-picked, spring-cycle leaves from *nonfruiting* terminals levels of 1.2 per cent of potassium in the dry matter for Hamlin and 1.5 per cent of potassium for Valencia oranges are best for maximum total solids in the fruit. However, too much potassium can decrease solids, as has been shown by Willson and Arey (1959). Also to be borne in mind is the fact that there are significant year-to-year variations in trees receiving the same treatment. This was noted by Chapman and Brown (1950), Deszyck and Koo (1957), and Willson and Arey (1959).

Potassium in Other Plant Parts.—Chapman and Brown (1950) reported analyses of wood, bark, roots, and fruit in relation to potassium status. Although all plant parts showed differences in relation to supply, they found that leaves were probably best used for diagnosis and fertilizer guidance work. Haas (1948*a*, 1948*b*, 1949*b*) has reported much data on flowers, leaves, rootlets, and fruit composition. Koo (1963) reported that the potassium of fruit reflected well the varying potassium status of trees.

Causes of Potassium Deficiency.—Potassium apparently performs many functions in the plant. It is an activator of enzymes, promotes growth in meristem tissues, neutralizes organic acids, and activates stomata opening and closing. When potassium is lacking, respiration increases; carbon dioxide assimilation decreases; carbohydrates first increase, then decrease; proteins decrease; amino acids increase; and mineral nutrients such as calcium, magnesium, iron, phosphorus, and nitrogen increase.

As with other elements, it is apparent that deficiency can result from unbalanced nutrient conditions within the plant and failure of plant roots to secure enough potassium from the soil or at a pace (in periods of maximum need) sufficient to produce best performance.

A complete account of what is known on the subject cannot be given here, and only a few soil factors will be mentioned.

Potassium exists in soil primarily in silicate mineral forms: (1) a part is easily exchangeable, being located on the platy surfaces and broken edges of a number of clay minerals, and (2) a part is not readily exchangeable or in "fixed" form, being located in less accessible parts of clay minerals. The mineralogical makeup of soils is an important factor in the distribution of potassium between exchangeable and nonexchangeable forms.

While few cases of a *marked* field deficiency of potassium on citrus have come to light, experience with many other plants shows generally that the most important cause of potassium deficiency in plants is a low content of easily exchangeable potassium and/or a very low total content of potassium in the soil. Plants can get some potassium from "nonexchangeable or difficultly exchangeable forms" (and this varies, too, depending on the kind of mineral clay which holds the potassium in "difficultly exchangeable" forms),

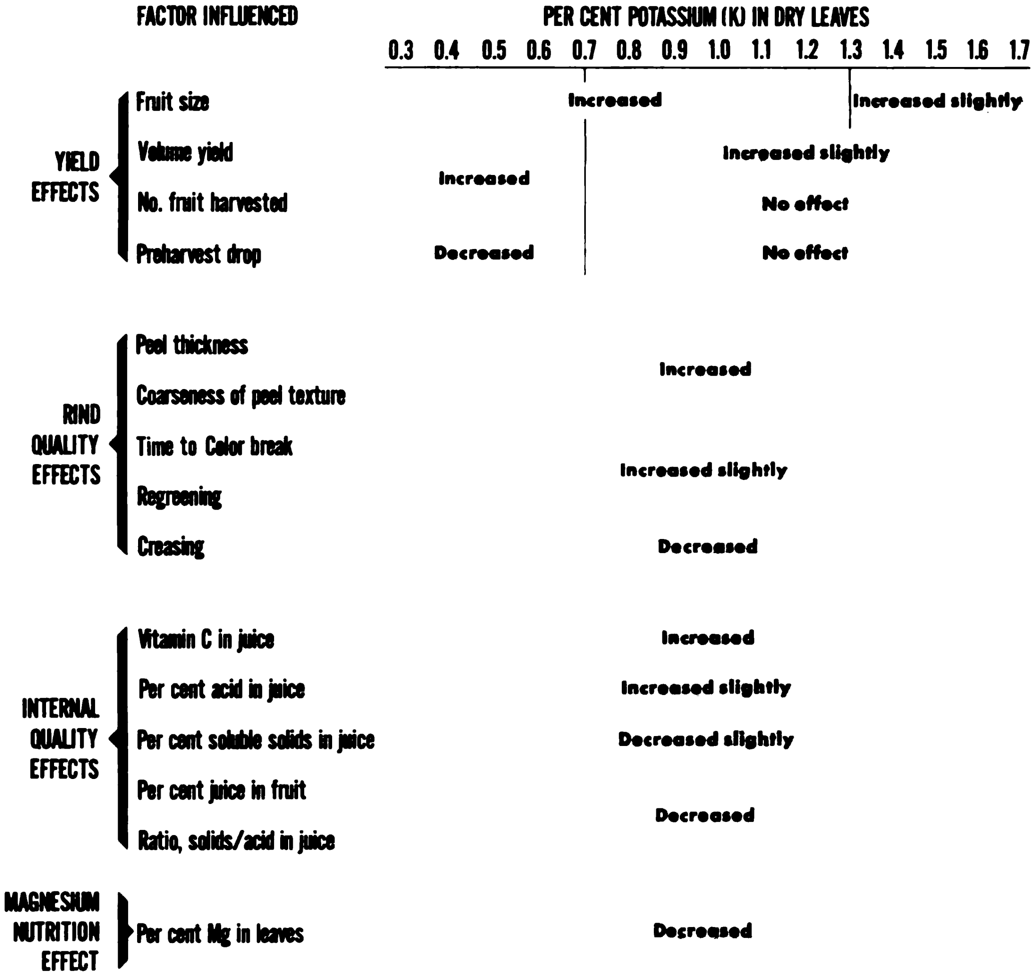

Fig. 3–58. Influence of the percentage of potassium in five- to seven-month-old bloom-cycle leaves from nonfruiting shoots upon factors of yield, rind quality, internal quality, and magnesium nutrition of oranges. (From Embleton, Jones, and Platt, 1967.)

but in many soils the difficultly exchangeable or fixed potassium cannot be obtained at a rapid enough rate to meet plant needs. It is, therefore, the more readily obtainable, exchangeable potassium which constitutes the important immediate source for plants. Exchangeable and nonexchangeable potassium in soils is in equilibrium, which is influenced by moisture content, plant withdrawal rates, temperature, and other factors. A good review of the subject has been given by Reitemeier (1951), and more recent work by Mortland and Ellis (1959), Mortland (1961), and Lai and Mortland (1961), has contributed to a better understanding of the mechanisms involved in potassium-diffusion processes in soils.

The calcium and magnesium status of soils is of undoubted importance in the potassium nutrition of citrus. Reuther, Smith, and Specht (1949), in a leaf analysis survey of citrus in Florida, Texas, Arizona, and California, noted that the potassium leaf levels were prevailingly low in California and Arizona compared with Florida. Among the soil differences which might account for this are the much higher calcium and magnesium contents of California and Arizona soils.

Fudge (1946) and many others have noted that increased calcium and magnesium in soils will decrease potassium uptake to some extent. Welch and Scott (1961) have shown that the ammonium ion can reduce the release of difficultly exchangeable potassium to plants.

Potassium has long been associated with

the water economy of plants. Its availability in soils is decreased by lack of soil moisture, and a good supply of potassium in plants tends to offset to some extent the effects of drought. Thus, lack of moisture in the soil increases potassium need, both through effects in the soil and in the plant. Increasing potassium levels in tissues of citrus have been shown to be associated with increasing succulence (Smith *et al.*, 1953).

Prolonged cropping will gradually deplete the available potassium of soils originally well supplied. In a fifteen-year lysimeter experiment, where the only fertilizer was calcium nitrate to provide 200 pounds of nitrogen per acre per year, Broadbent and Chapman (1950) found that there had been a net loss of 3,552 pounds of potassium and a net gain of 6,189 pounds of calcium on a per acre basis. Under these conditions, it is plain that potassium would sooner or later be needed.

Effect of Rootstock.—There is insufficient data from field experiments and controlled cultures to assess the rootstock effect in relation to potassium adequacy, but differences in potassium composition of flowers from Eureka lemon and Valencia orange grown on various rootstocks were reported by Haas (1948*b*). He found that high potassium in flowers of both species occurred where tangelo and grapefruit stock were used, as compared with lower values with sour orange and rough lemon. Values ranging from 1.1 to 1.8 per cent of potassium in the dry matter were found. Further analyses of fruit peel, leaves, and rootlets of oranges, grapefruit, and lemon for potassium, calcium, magnesium, and phosphorus were reported by Haas (1948*a*). In peel, potassium was commonly high when trees were grown on trifoliate stock, and low on rough lemon. There were marked differences in composition generally in relation to rootstock, indicating an important effect on nutrition. However, the relation of these findings to soil potassium levels and deficiency and excess effects requires further exploration.

Smith, Reuther, and Specht (1949) found that grapefruit and sweet orange roots induced higher leaf potassium than citrange roots. Gorton, Cooper, and Peynado (1954) found that Webb Red Blush grapefruit absorbed less potassium (and more calcium) when grown on Cleopatra mandarin rootstock than when grown on sour orange rootstock.

Relation of Potassium Deficiency to Disease Resistance.—Although the author is unaware of specific data for citrus, there are many diseases that are aggravated by a lack of potassium. A review and discussion of these diseases and other soil fertility conditions affecting plant diseases is given by McNew (1953) and Chapman (1964). Some of the diseases aggravated by lack of potassium are angular leaf spot of tobacco *(Pseudomonas tabaci)*, "take-all" disease of wheat *(Ophiobolus graminis)*, cotton wilt *(Fusarium vasinfectum)*, powdery mildew of cereals *(Erysiphe graminis)*, cereal rusts *(Puccinia glumarum, P. graminis*, and others), tobacco mosaic virus, and cabbage yellows *(Fusarium oxysporum, F. conglutinans)*.

Control of Potassium Deficiency.—While it is possible to easily correct moderate to acute potassium deficiency with applications of potassium sulfate or potassium chloride (1 to 2 pounds of K_2O equivalent per tree), it requires time on many soils to substantially increase leaf levels from soil applications. The author and his colleagues made yearly leaf analyses of a large number of California orchards where 5 to 10 pounds of potassium sulfate was broadcast yearly per tree. A summary of these data are presented in table 3–33. They show that in many cases rather large amounts of potassium fertilizer must be applied over a period of years to substantially increase potassium levels in the tree.

In a citrus fertilizer experiment at Riverside, California, analyses of leaves from plots receiving 1 pound of K_2O per tree from potassium sulfate from 1927 to 1940 showed an increase in leaf potassium from values of around 0.62 to 0.65 to 1.07 to 1.18 per cent in dry matter. The soil was a rather compacted, sandy loam receiving about 30 to 36 inches of irrigation water a year and an annual average rainfall of about 11 inches. Trees in plots receiving dairy manure at rates supplying 3 pounds of nitrogen per tree showed potassium values in leaves up to 1.56 per cent. Because of the ameliorating effects of organic matter on soil structure, it appears likely that with improved permeability to water, potassium penetrates into dense citrus root areas more rapidly when organic matter is used as a source of potassium or along with inorganic sources. The author has repeatedly noted marked citrus feeder root development in and around organic manure masses in soils.

Recommendations of desirable annual application of potassium on mature orchards vary from 1 to 4 pounds of K_2O per tree per year or from about 100 to 400 pounds per acre. The latter rate is higher than needed for maintenance, once

Table 3–33

EFFECT OF BROADCAST POTASH APPLICATION ON POTASSIUM CONTENT OF CITRUS LEAVES

Age and Variety	Soil Type	General Fertilizer Treatment	Treatment and Leaf Analyses					
			Date Treatments Begun	Pounds of Potassium Per Tree Applied Prior to Leaf Sampling	Per Cent Potassium in Dry Matter	Date of Leaf Sampling	Pounds of Potassium Per Tree Applied Prior to Leaf Sampling	Per Cent Potassium in Dry Matter
27-year-old Valencia orange	San Joaquin loam	Manure and ammonium sulfate prior to 1942	June, 1942	0.0	0.59	June, 1945	0.0	1.13
				9.0	0.70		22.0	1.62
40-year-old Valencia orange	San Joaquin loam	$NaNO_3$ and $(NH_4)_2SO_4$	June, 1942	0.0	0.49	June, 1945	0.0	1.18
				9.0	0.52		18.0	1.43
40-year-old navel orange	San Joaquin loam	$NaNO_3$ and $(NH_4)_2SO_4$	June, 1942	0.0	0.91	June, 1945	0.0	0.69
				9.0	0.93		18.0	1.28
Navel orange	San Joaquin loam	$(NH_4)_2SO_4$, $Ca(NO_3)_2$, plus straw	June, 1942	0.0	0.65	June, 1945	0.0	1.04
				9.0	0.83		22.0	1.31
9-year-old lemon	Loam	$(NH_4)_2SO_2$ and $NaNO_3$	June, 1942	0.0	0.37	June, 1945	0.0	0.26
				13.0	0.43		18.0	0.34
8-year-old lemon	Sandy loam	$NaNO_3$, $(NH_4)_2SO_4$, and Cal-Nitro	June, 1942	0.0	0.43	June, 1945	0.0	0.52
				4.5	0.47		13.0	0.55
Young lemon	Sandy loam	Unknown	June, 1942	0.0	0.62	June, 1945	0.0	0.50
				4.5	0.66		13.0	0.52
45- to 50-year-old Valencia orange	Hanford sandy loam	$NaNO_3$ and manure	June, 1942	0.0	0.68	June, 1945	0.0	1.04
				4.5	0.71		13.0	1.11
Lemon	Hanford sandy gravel loam	$NaNO_3$	Sept., 1941	0.0	0.78	June, 1943	0.0	0.56
				20.0	0.78		21.0	0.57
25-year-old lemon	Hanford sandy gravel loam	Unknown	Sept., 1941	0.0	0.62	June, 1943	0.0	0.64
				21.0	0.69		21.0	0.69
9-year-old Valencia orange	Hanford sandy loam	Tankage and $(NH_4)_2SO_4$ in 1942–43 only	June, 1940	0.0	0.59	June, 1945	0.0	0.94
				9.0	0.66		22.0	1.07
25-year-old Valencia orange	Ramona sand	Chicken manure and $(NH_4)_2SO_4$	June, 1941	0.0	0.62	June, 1943	0.0	0.77
				9.0	0.68		18.0	0.87
14-year-old Valencia orange	Ramona sandy loam	$Ca(NO_3)_2$, $(NH_4)_2SO_4$, and manure	Oct., 1941	0.0	0.67	June, 1944	0.0	0.63
				9.0	0.73		18.0	0.90
Old lemon	Ramona loam	Unknown	Aug., 1941	0.0	0.66	June, 1944	0.0	0.86
				21.0	0.77		21.0	0.98
42-year-old Valencia orange	Placentia loam	Manure and $(NH_4)_2SO_4$	Sept., 1941	0.0	0.61	June, 1943	0.0	0.72
				13.0	0.64		18.0	0.91
40-year-old Valencia orange	Yolo gravelly loam	Manure and Cal-Nitro	Sept., 1941	0.0	0.92	June, 1942	0.0	0.53
				21.0	0.94		21.0	0.61
60-year-old navel orange	Placentia loam	Manure and $(NH_4)_2SO_4$	Sept., 1941	0.0	0.86	June, 1944	0.0	0.95
				13.0	0.85		18.0	1.11

a low potassium condition has been corrected.

Table 3–13 on page 136 indicates that 20,000 pounds of fruit will remove about 63 pounds of K_2O. Heavier average yields would require proportionally more potassium.

As stated earlier, the clay fraction of soils fixes potassium both in exchangeable and nonexchangeable forms; potassium movement downward is usually slow, and leaching losses are negligible. In a loam soil of 7.0 meq/100 gm exchange capacity, Broadbent and Chapman (1950) found that yearly losses by leaching of potassium from a 4-foot depth of soil over a fifteen-year period ranged from less than 1 pound to not much over 2 pounds of potassium per acre per year.

In a sandy Florida citrus soil of very low clay content, Kime (1944) found that potassium moved quite readily. In this kind of soil, it would be very easy to correct potassium deficiency.

In a recent study of the possible use of foliar sprays, Page, Martin, and Ganje (1963) found that the potassium of citrus leaves could be substantially increased by the use of 0.5*M* potassium nitrate (42 pounds per 100 gallons). They used Triton B-1956 (0.25 per cent v/v) spreader. They applied approximately 3 gallons of the potassium nitrate spray per tree per treatment to ten-year-old Valencia oranges in May and June and were able, with one spray treatment, to increase the potassium content of the leaves by 0.32 per cent in the dry matter. Only very minor leaf injury occurred. The calcium, magnesium, and sodium contents of the leaves were not affected.

More work is under way to determine the safest and most effective method of using potassium sprays, but the foregoing results show that potassium deficiency and low potassium status can be quickly remedied by the use of one or more foliar spray treatments. Treatments can then be followed by maintenance soil applications.

Potassium Excess

Potassium is readily absorbed by citrus and other plants, and characteristic toxicity symptoms have been produced under controlled culture conditions. However, under field conditions, because of the high fixing power of soils for this element, high concentrations of potassium seldom are encountered in soil solutions; hence, absorption of this element in amounts sufficient to decrease growth and produce leaf injury has not been reported. However, as noted previously, adverse effects on fruit quality (such as fruit coarseness, delayed maturity, elevated acid, and lowered total solids) are possibilities and have been noted in Florida (Koo, 1963; and others). Under these conditions, tree growth and fruit yields are not impaired, but fruit quality is reduced.

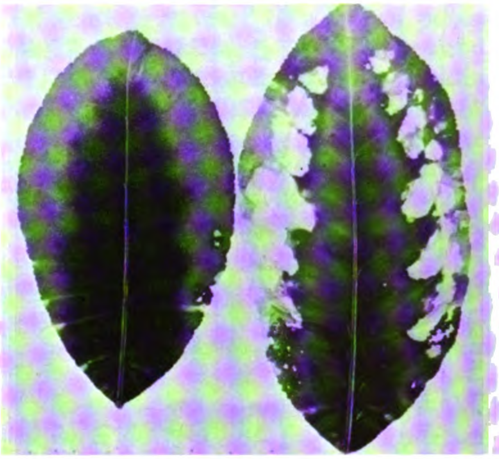

Fig. 3–59. Lemon leaves showing marginal yellowing and burning produced by excess potassium in the nutrient solution. The leaves contained 4.95 per cent of potassium in the dry matter.

Another more common effect of increasing potassium accumulation in soils is the production of magnesium deficiency. This has occurred rather widely to a moderate degree on citrus in California and has been noted with a very large number of other crops in many parts of the world.

There is also evidence that in some soils excess potassium interferes with manganese and zinc absorption.

In controlled culture work, Chapman and Brown (1942) found that orange trees grown in solutions of high-potassium content were more susceptible to brown-rot gummosis *(Phytophthora parasitica)*.

Effects of Potassium Excess.—In water- and sand-culture experiments, the author produced a characteristic type of leaf burn from excessive absorption of potassium (fig. 3–59).

In some cases, water-soaked areas developed in old leaves, and these lesions soon became necrotic. In other experiments, a marginal yellowing followed by necrosis took place. The potassium in these burned leaves was very high—nearly 5 per cent of potassium in the dry matter.

Growing trifoliate citrus seedlings in soils containing increased amounts of exchangeable potassium, Martin, Bitters, and Ervin (1959) found that growth was decreased when the percentage of potassium as of total bases exceeded 23 per cent. The potassium in the leaves of these plants exceeded 3 per cent of potassium in the dry matter. In similar experiments with young lemon trees, Jones, Martin, and Bitters (1957) produced depressed growth in a loam soil when exchangeable potassium was at 23 per cent.

From the evidence available, the likelihood of direct foliage and tree injury from excess potassium accumulation in the soil (except under some alkali soil conditions) is remote. However, unbalanced nutrition (due to effects on magnesium, calcium, manganese, and zinc absorption) does occur widely, and the possibilities of significant fruit quality impairment (rough, thick rind, lower juice, and lower total solids) are very real. Leaf analyses for potassium, calcium, and magnesium plus visual symptoms (supplemented if need be by soil analysis and evaluation of soil-management practices) offer the best means of determining whether potassium is excessive or moving in that direction. The leaf analysis values for potassium reported in table 3–32 are a useful guide, and reference to leaf analysis standards (Chapman, 1949, 1960; Reuther *et al.*, 1958) will prove helpful in evaluating the total nutritional situation.

SULFUR (S)

Sulfur Deficiency

Effects of Sulfur Deficiency.—This section deals only with sulfur as an essential plant nutrient and not with its use in ameliorating soil alkali or alkaline soil conditions.

Because of the widespread use of sulfur-containing materials (such as ammonium sulfate, potassium sulfate, heavy metal sulfates, superphosphate, gypsum, animal manures, insecticides, and fungicides), the presence of sulfur as sulfate in irrigation waters, and sulfur's occurrence in some areas as an air pollutant, sulfur deficiency of citrus has never been encountered under field conditions.

In a study of the sulfur balance problem in plots at the Woburn Experimental Station in England, Mann (1955) concluded that under normal rural conditions sulfur from rain and other atmospheric sources is sufficient to maintain the sulfur content of the soil. This study was conducted in an area where barley had been grown for fifty years. However, in nonirrigated areas of both the semiarid and humid regions, sulfur deficiency has been uncovered by investigators in many crops and soil types through the world, especially in the Northern Hemisphere: Powers (1923); Alway (1940); Conrad (1950); Coic and Lefebvre (1960); Harper (1959); Kramp (1958); McClung and de Freitas (1959); Storey and Leach (1933); Jordan and Bardsley (1958); Bardsley and Jordan (1957); Harward, Chao, and Fang (1962); Chittenden and Dodson (1961); Kamprath, Nelson, and Fitts (1957); Ollagnier and Prevot (1957); and Walker (1957). Freney, Barrow, and Spencer (1962) recently reviewed various aspects of the sulfur problem, such as the forms of sulfur in soil, accession from the air and rain, losses from soils, transformations in soils, and methods of diagnosing sulfur status.

The chief reservoir of soil sulfur is organic matter. Relatively lesser amounts are found as sulfides in rocks. In some soils, especially in the arid and semiarid regions, sulfates may be deposited by water and accumulated from the oxidation of organic forms and sulfides, and thus provide an additional supply. These sulfate deposits are especially likely to be found in subsoil horizons. Also, there is some evidence that calcium sulfate may be cocrystallized as an impurity in calcium carbonate (Williams and Steinbergs, 1962), and there may be insoluble barium and strontium sulfates in some soils. Sulfate also may be absorbed by some clay minerals in very acid soils (Ensminger, 1954; Kamprath *et al.*, 1957).

The air contains various sulfur impurities. Even in rural areas, rainfall may bring down annually from 1 to 12 or more pounds of sulfur per acre, depending principally upon location with respect to industrial centers and the seashore.

As stated earlier, sulfur deficiency of citrus has never been reported in the field. Haas (1936*d*) described a type of leaf chlorosis on young Valencia orange trees growing in sand culture. Later, Chapman and Brown (1941*b*) produced sulfur deficiency symptoms on young, bearing, navel orange trees growing outdoors in soil cultures. A pronounced yellowing of the new growth (the leaves were uniformly yellow as in nitrogen deficiency) was the first symptom. Subsequent growth cycles were yellower, the leaves were smaller, and premature abscission of many affected leaves occurred. A tendency for the production of an abundant but weak bloom was noted. Weakened shoots died back, but no gumming of the bark took place. The fruits produced were often somewhat undersized and misshapen, and they failed to develop the orange color characteristic of navel oranges

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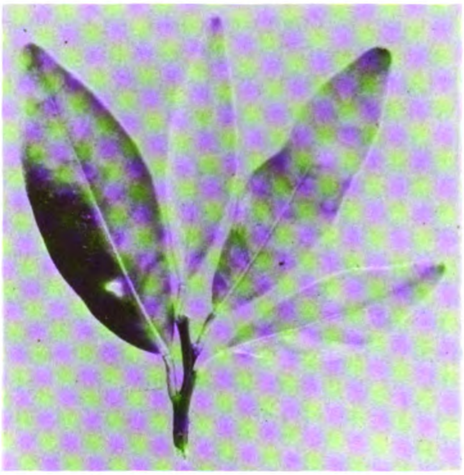

Fig. 3–60. Sulfur-deficient leaf showing a green area where a Roach wick containing sulfate was inserted into the leaf. This is a simple means of determining whether the yellow foliage is due to sulfur or nitrogen deficiency.

grown in the area of Riverside, California. The rinds were abnormally thick, and in severely affected fruits the juice sacs were shriveled and dry. Where the symptoms were less pronounced, granulation of some sections of the fruit was noted. All parts of the tree were abnormally low in sulfur content, with nitrogen above average. Guest and Chapman (1946, unpublished data) found that leaf microinjections of calcium, sodium, or potassium sulfates caused the leaf to become green in an area surrounding the point of treatment (fig. 3–60). Some effects of sulfur deficiency are shown in figures 3–61 through 3–64. Recovery of these plants was rapid when sulfate was added to the nutrient medium.

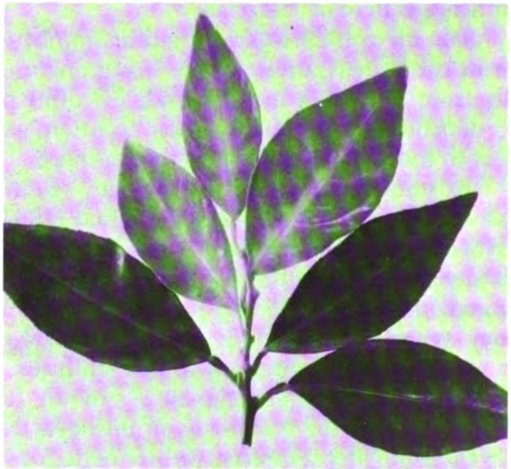

Fig. 3–61. Shoot from a sulfur-deficient navel orange tree, showing yellow, new-cycle leaves which stand in sharp contrast to the older, green leaves. In many instances, the midrib is somewhat more yellow than the rest of the leaf. This type of growth is especially prominent in the earlier stages of sulfur deficiency. The yellow, sulfur-deficient leaves are similar in appearance to nitrogen-deficient leaves.

Table 3–34 presents data showing the inorganic composition of various parts of sulfur-deficient and healthy trees. The comparative sulfur and nitrogen contents of leaves from trees deficient in these respective elements are shown in table 3–35.

Leaves from sulfur-deficient trees are low in sulfur, ranging from 0.05 to 0.13 per cent in the dry matter, whereas the nitrogen content is high. Commonly, leaves from nitrogen-deficient plants are low in nitrogen and high in sulfur. Since there is considerable similarity in the appearance of trees lacking sulfur and nitrogen, leaf analysis is an effective means of differentially diagnosing the two conditions.

Rasmussen and Smith (1958) have reported the effects of leaf age on the sulfur content of Valencia orange leaves, and their data are reproduced in table 3–36. The data show remarkably little change with leaf age. One-week-old leaves are slightly higher than those three weeks to eleven months old, and those at thirteen to fifteen months of age are only slightly lower.

Diagnosis of Sulfur Status.—Since sulfur deficiency of citrus has not been encountered in the field, no special effort has been made to develop diagnostic techniques. However, the good correlation noted by Chapman and Brown (1941*b*), Aldrich, Buchanan, and Bradford (1955), and others between the total sulfur of leaves and sulfur status indicates that leaf analysis and visual symptomatology are satisfactory means of determining current sulfur status. There is quite a little evidence with other plants that sulfate-sulfur is a better index of sulfur status than total sulfur (Eaton, 1966), and this may prove true for citrus.

The increasing incidence of sulfur deficiency in soils has led to considerable research on the development of soil test methods. Phosphate-extraction methods of Ensminger (1954), Fox, Olson, and Rhoades (1964), and Bardsley and Lancaster (1960) appear promising. While leaf analysis and other plant criteria can reveal current sulfur status, soil availability tests make possible predic-

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Table 3–34

COMPARATIVE INORGANIC COMPOSITION OF PARTS OF SULFUR DEFICIENT AND HEALTHY NAVEL ORANGE TREES

Part of Tree and Condition	Constituents of Dry Matter (In Per Cent)								
	Ash	Ca	Mg	K	Na	Cl	N	P	S
Young leaves:									
Sulfur-deficient	16.02	3.56	0.22	3.40	0.210	0.35	4.90	0.50	0.050
Healthy	14.23	4.34	0.12	1.55	0.020	0.39	3.38	0.18	0.230
Old leaves:									
Sulfur-deficient	15.40	5.05	0.18	0.81	0.100	0.42	5.30	0.34	0.130
Healthy	22.80	8.17	0.09	0.80	0.040	0.35	1.70	0.11	0.260
Twig bark:									
Sulfur-deficient	12.25	4.32	0.13	0.34	0.010	0.12	3.03	0.10	0.010
Healthy	15.47	5.22	0.12	0.62	0.005	0.14	1.65	0.28	0.270
Twig wood:									
Sulfur-deficient	5.31	1.89	0.06	0.15	0.010	0.09	0.85	0.01	0.004
Healthy	4.12	1.26	0.08	0.24	0.004	0.14	0.72	0.22	0.120
Trunk bark:									
Sulfur-deficient	12.83	4.23	0.25	0.29	0.005	0.07	2.12	Trace	0.010
Healthy	13.15	4.40	0.35	0.66	0.005	0.11	1.64	0.24	0.180
Trunk wood:									
Sulfur-deficient	3.32	1.10	0.07	0.17	0.004	0.12	0.73	Trace	0.010
Healthy	2.49	0.69	0.08	0.21	0.003	0.14	0.60	0.16	0.110
Root bark:									
Sulfur-deficient	8.75	2.56	0.12	0.61	0.006	0.35	2.66	0.22	0.040
Healthy	11.00	3.26	0.18	0.75	0.020	0.40	2.15	0.24	0.200
Root wood:									
Sulfur-deficient	1.78	0.52	0.09	0.09	0.009	0.14	0.70	0.06	0.008
Healthy	2.64	0.73	0.09	0.18	0.005	0.12	0.66	0.16	0.080
Fine roots:									
Sulfur-deficient	13.73	4.04	0.21	0.54	0.010	0.39	2.81	0.30	0.080
Healthy	28.23	4.46	0.22	0.59	0.040	0.32	1.95	0.25	0.140

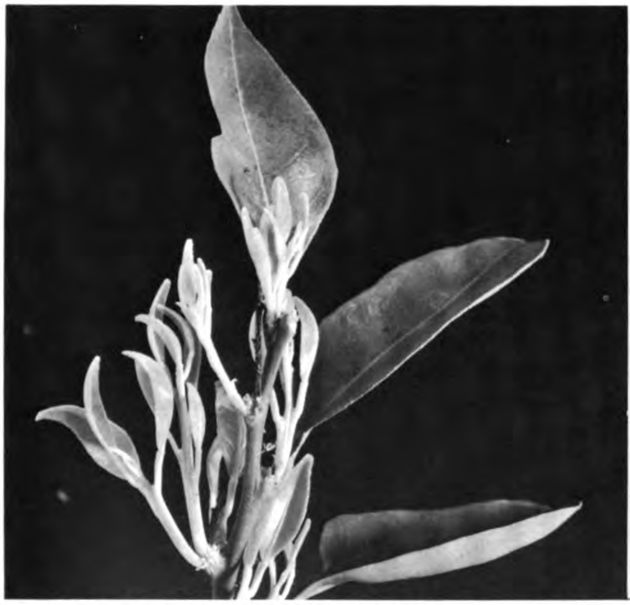

Fig. 3–62. Twig of sulfur-deficient orange tree showing weak, yellow, and multiple young shoots.

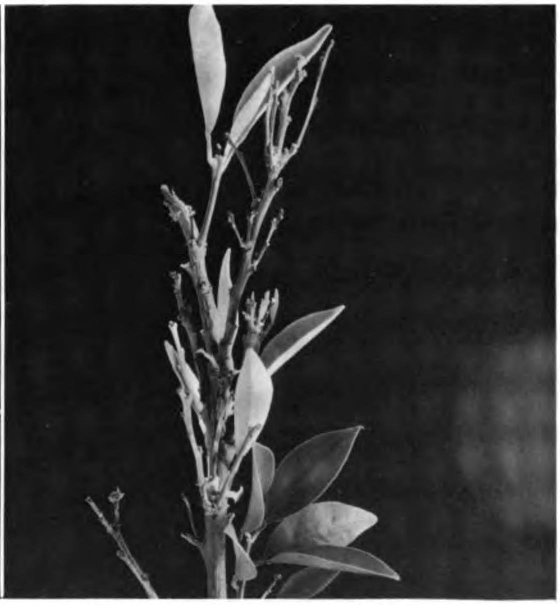

Fig. 3–63. Acute stage of sulfur deficiency, showing dieback, multiple buds, and weak and sparse yellowed new growth on an orange shoot.

Table 3–35

SULFUR AND NITROGEN CONTENTS OF LEAVES FROM HEALTHY AND YELLOWED CITRUS PLANTS

Source, Age, and Character of Leaves Tested	Composition of Leaves in Percentage of Dry Matter	
	Total Sulfur	Total Nitrogen
Sulfur-deficient sweet orange seedlings grown in greenhouse:		
Young terminal yellow leaves	0.075	3.88
Old green leaves	0.120	3.31
Healthy sweet orange seedlings grown in greenhouse:		
Young terminal green leaves	0.260	3.46
Old green leaves	0.220	3.15
Sulfur-deficient navel orange trees grown in Sierra loam soil:		
Young terminal yellow leaves	0.096	2.54
Old green leaves	0.129	2.44
Nitrogen-deficient navel orange tree grown in Sierra loam soil:		
Young terminal yellow leaves	0.189	1.12
Old yellow leaves	0.320	1.13
Healthy sulfur-treated navel orange tree grown in Sierra loam soil:		
Young terminal green leaves	0.202	2.15
Old green leaves	0.320	1.66

Table 3–36

SULFUR CONTENT OF VALENCIA ORANGE LEAVES IN RELATION TO AGE OF LEAVES

Leaf Age	Sulfur in Dry Matter (Per Cent)
1 week	0.35
3 weeks	0.31
2 months	0.29
3.5 months	0.33
5 months	0.32
6.5 months	0.32
8.5 months	0.32
9.5 months	0.31
11 months	0.33
13 months	0.28
14 months	0.29
15 months	0.29

Source: Rasmussen and Smith (1958).

tions of sufficient reliability to warrant field testing.

Causes of Sulfur Deficiency.—As stated earlier, the main source of sulfur in soils is in the organic fraction,[3] with lesser amounts present as sulfides and sulfates. The nonsulfate forms gradually become converted to soluble sulfates, and, with crop removal on the one hand and leaching losses on the other, sulfur deficiency would soon develop but for the addition of sulfate-containing fertilizers, organic manures, pesticides, fungicides, irrigation waters, or additions from the atmosphere.

Though specific cases of sulfur deficiency of citrus in the field have not yet been reported, they should be looked for in nonirrigated citrus areas and especially on soils low in organic matter, such as many of those found in the tropics and high rainfall regions.

As to factors affecting sulfate absorption and utilization, some data with citrus and other plants have been reported. These data are briefly reviewed in the following subsection.

Effects of Other Nutrients on Sulfate Absorption.—In carefully controlled nutritional experiments, Chapman and Liebig (1940) found that increasing nitrate supplies did not depress sulfate absorption. Rasmussen and Smith (1958) got similar results with Valencia orange on rough lemon rootstock. The sulfur content of leaves of trees fertilized at three rates with ammonium nitrate (0.75, 1.5, and 3 pounds of nitrogen per tree) showed, respectively, 0.33, 0.34, and 0.34 per cent of sulfur in the dry matter. However, under conditions of sulfate excess, Foote and McElhiney (1937) found that extra nitrogen fertilizer seemed to decrease sulfur-excess effects. Haas and Thomas (1928) noted similar effects in pot tests.

Jones *et al.* (1963) are of the opinion that under conditions of high sulfate extra nitrogen is helpful. Perhaps the true explanation of these somewhat conflicting results is that when nitrogen is somewhat deficient, sulfate tends to accumulate in leaves and produce the typical, excess-sulfur leaf pattern. Chapman and Liebig (1940) noted a higher leaf sulfur content in nitrogen-starved sweet orange seedlings than in those receiving ample nitrate.

With regard to phosphorus, Rasmussen and Smith (1958) likewise found that rates of phosphate fertilizer, ranging from 0 to 4.5 pounds of

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[3] The sulfur in the organic fraction may be tied up as sulfate covalently bound to polysaccharides, as sulfate esters of phenols, or in cystine and methionine forms (Freney, 1961).

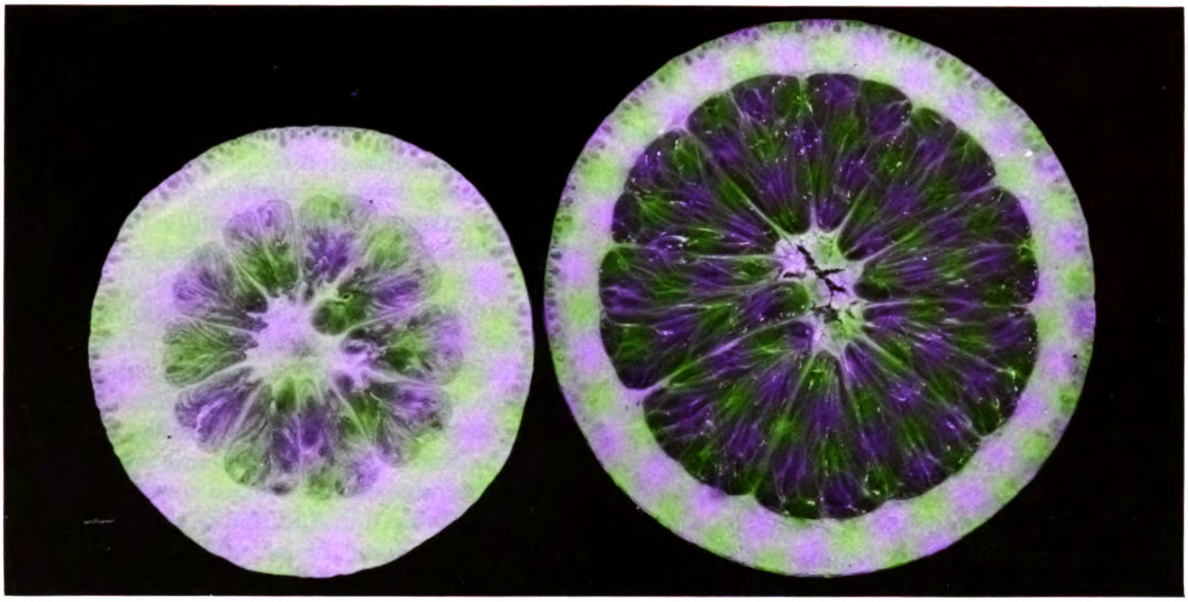

Fig. 3–64. Cross-sections of mature, sulfur-deficient (left) and healthy (right) navel oranges. Note the thickened rind and shriveled juice vesicles of sulfur-deficient fruit. This is a somewhat extreme case; not all fruits from sulfur-deficient trees are so adversely affected.

phosphate (P_2O_5) per tree and under conditions of equal sulfate supply, did not have any effect on sulfate uptake. Chapman and Brown (1941*a*) got essentially the same results with phosphate in their fertilizer tests with navel oranges growing under phosphatic fertilization.

Effects of Soil Moisture.—Dutt (1962*b*) found with sugar cane that rain and irrigation produced beneficial effects on sulfur-deficient plants and drew the inference that drought reduces soil-sulfur absorption. This appears plausible, since similar findings have been noted with phosphorus, potassium, and other nutrients. Among other things, the nutrient diffusion rate in soil films is reduced as moisture decreases.

Effect of Rootstock.—Though not much data with citrus are available on sulfur uptake and leaf composition as affected by rootstock, Rasmussen and Smith (1958) found with Valencia and Parson Brown scions on grapefruit and rough lemon rootstocks, respectively, that under the same culture conditions the leaf sulfur of the two scions was 1.14 and 0.91 per cent of sulfur in the dry matter, as compared with 0.43 and 0.41 per cent of sulfur for scions grown on rough lemon stock. Since marked effects of rootstock on leaf content of boron, potassium, nitrogen, chlorine, and other elements occur, it is reasonable to assume that sulfate uptake would also be affected. However, much more work is needed in this respect to determine which rootstocks would be most effective, especially where sulfate is in excess. (See the salinity section, p. 243, for a more complete account of rootstock effects on salt uptake.)

Control of Sulfur Deficiency.—With other crops, control has been easily accomplished by soil application of gypsum and other sulfate-containing fertilizers (McKell and Williams, 1960). Elemental sulfur can also be used. All sulfate forms are sufficiently mobile that no problem of movement to roots is involved, and gypsum is of low enough solubility that large applications can be made without much danger of toxicity to citrus. Correction lasting for a period of years can be obtained with gypsum.

Leaves will absorb sulfate from foliar applications, and though no field data with citrus are available, Dutt (1962*a*, 1962*b*) has reported that foliar correction of sulfur deficiency on jute and sugar cane was achieved with sprays of 0.08*N* sulfuric acid and 0.3 per cent solutions of copper or zinc sulfates.

Rates for soil application on sandy soils, which have proved sufficient for correction of sulfur deficiency of crops such as clover, are as low as 16 pounds of sulfur per acre as gypsum (Neller and Bartlett, 1959).

Sulfur Excess

Only the nutritional aspects of sulfate toxicity will be dealt with in this section. The toxicity of elemental sulfur to foliage and fruit when used as a fungicide and insecticide will not be covered. Turrell (1950), Turrell and Chervenak (1949), and Turrell and Boyce (1953) have investigated and reported on this subject in great detail.

Sulfate and chloride, and to a lesser extent bicarbonate and nitrate, are the principal anions which make up the salt constituents of alkali and saline soils. The subject of alkali and salinity is treated in a later section. Only brief mention of the specific effects of excess sulfate will be taken up here.

An element of uncertainty exists with respect to the specific effects of the sulfate ion per se on citrus. The associated cation may be of greater importance in determining toxicity than sulfate. However, it can be stated in general that the sulfate ion is much less toxic and not taken up as readily by citrus roots as the chloride ion, and with respect to the associated cation, sodium sulfate is much more toxic than calcium or magnesium sulfates. In fact, because of its limited solubility, excess gypsum ($CaSO_4 \cdot 2H_2O$) appears not to materially limit citrus growth or production where supplies of nitrogen and other elements are adequate. Many of the Imperial Valley soils of California contain excess solid-phase gypsum, and (where not complicated by a high water table or other soluble salts) excellent, high-producing orange and grapefruit orchards can be found. However, under some conditions, excess calcium sulfate can be injurious.

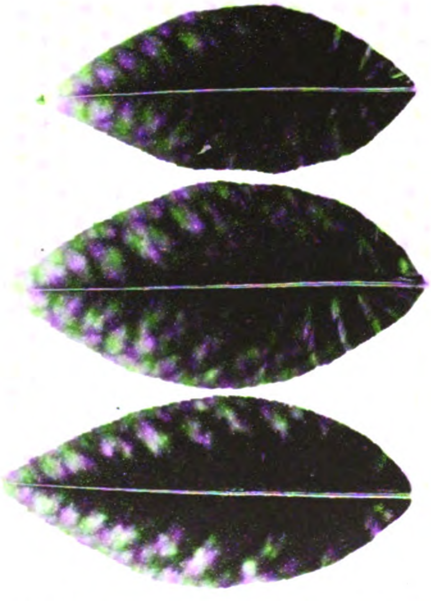

Fig. 3–65. Lemon leaves showing mottling from absorption of excessive sulfate. This type of pattern closely resembles injury from excess boron, but it can usually be differentiated by a leaf analysis for boron and sulfur. If it is due to the latter only, the sulfur level will usually be > 0.50 per cent in the dry matter.

Haas and Thomas (1928) grew budded lemon trees in 10-gallon crocks of quartz, to which 1 pound of calcium sulfate was added. Nutrient solutions of increasing calcium nitrate content were added to various cultures. Under low-nitrogen conditions, the leaves became mottled and yellowish and showed 1.57 per cent of total sulfur in the dry matter. Addition of extra nitrate decreased the amount of leaf yellowing and mottling, but did not completely prevent it. Injured leaves dropped prematurely. Leaf patterns characteristic of excess sulfate are shown in figure 3–65.

In carefully controlled sand-culture experiments with sweet orange seedlings involving nitrate and sulfate variables, no growth depression occurred from the presence of 20 meq/l of sulfate (derived from calcium, magnesium, and potassium) even at low-nitrogen concentrations (Chapman and Liebig, 1940).

Aldrich *et al.* (1955) grew Eureka lemons (on sweet orange rootstock) in 3-gallon pots of an alkaline soil treated with sufficient sulfur to increase soil acidity to values of approximately pH 7, 6, 5, and 4, respectively. In practically all cases, increased sulfur additions and soil acidity decreased growth. The chief cause appeared to be excessive sulfate absorption by the plant and resulting leaf injury. Leaf patterns identical to those produced by Haas and Thomas (1928) were noted, and leaf values for sulfur varied from 0.20 to 1.47 per cent of sulfur in the dry matter. First signs of leaf patterns appeared when the sulfur content of the leaf reached 0.5 per cent. The sulfur treatments produced marked increases in the soluble calcium and sulfate content of saturation extracts, the latter reaching values up to 68.6 meq/l in one soil treatment. In this case, the soluble calcium, magnesium, and potassium values were 21.5, 23, and 6.8 meq/l, respectively. The total soluble salt content in some of these soils reached conductivity values of over EC 2.0 mmho/cm, and decreased growth might have been the result not only of

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excess sulfate uptake, but because of the salinity of the soil solution.

In a long-time fertility experiment at Riverside, sulfur was applied regularly to certain plots. From 1927 to 1949, 80 pounds of sulfur per tree (or an equivalent of 7,200 pounds per acre) were applied. Soil pH was markedly reduced in the first two feet of soil, with values somewhat less than pH 5 having been produced. In comparable treatments with less sulfur, the reaction varied from pH 6.4 to 7.2. Soluble salts (mostly calcium sulfate) were produced in the sulfur-treated plots, but yields were not affected by the sulfur treatment.

Zusman (1956) grew sweet lime and sour orange seedlings in pots of sandy soil treated with increasing amounts of sulfuric acid and produced thereby sulfates in amounts varying from 381 to 2,293 ppm sulfate (SO_4) in the soil. Top growth (rated at 100) at the 381-ppm sulfate level was reduced by 50 or more per cent at the highest sulfate level, and a leaf yellowing and browning starting at the periphery was noted similar to that produced by Haas and Thomas (1928). Total sulfur ranged from 0.35 to 0.64 per cent in sour orange leaves from trees growing in soils of increasing sulfate content, while total calcium in the leaves decreased as sulfur increased.

Control of Sulfur Excess.—Excesses of sulfate resulting from salinity increases in soils must be dealt with by better control of drainage and irrigation practices, as outlined under the section on salinity and alkali (p. 243).

As stated earlier, the use of extra nitrogen appears to help overcome excess sulfate in soils, especially under conditions where nitrogen may be deficient. Not enough is known about rootstock effects as yet, but the data of Rasmussen and Smith (1958) clearly indicate that orange trees on rough lemon stock may tolerate high sulfate better than when on grapefruit stock.

ZINC (Zn)

Zinc Deficiency

As with manganese and boron, early workers noted plant stimulation and responses to zinc and suggested that this element might be essential: Raulin (1863, 1869, 1870); Javillier (1908, 1912, 1914*a*, 1914*b*); Steinberg (1918, 1919); Mazé (1914, 1915); Sommer and Lipman (1926); and Sommer (1927, 1928). Final proof that zinc was an essential and indispensable element for citrus and other plants emerged from several related investigations in the 1930's. First there was marked correction of "little-leaf" of peaches and citrus "mottle-leaf" from massive soil applications of zinc-contaminated iron sulfate. When various zinc compounds alone were applied to the soil and as foliar sprays, there was spectacular recovery from these disorders (Chandler, Hoagland, and Hibbard, 1932, 1933, 1934, 1935; Johnston, 1933; and Parker, 1934, 1936, 1937*a*, 1938). This field work was followed by controlled water-culture experiments on apricot by Hoagland, Chandler, and Hibbard (1936), and on citrus by Chapman *et al.* (1937). In this controlled work, typical little-leaf of apricots and mottle-leaf of citrus were produced by reducing zinc contamination in culture solutions.

After the California field work, Mowry and Camp (1934) were able to correct "frenching" of citrus with zinc sprays in Florida. Thus ended a long period of investigational work into the cause of citrus mottle-leaf. For years this disorder had plagued citrus growers in California. While it was known to be worse on sandy and loamy soils (especially those derived from igneous rocks) than on heavier types, to be aggravated by fertilizer (such as sodium nitrate), and to be ameliorated by organic matter, still the disorder was a serious problem, and the basic cause was unknown until the aforementioned pioneer work of Chandler and others in California.

The discovery of its true cause and practical means of control is one of the classic examples of benefit to an industry and agriculture through

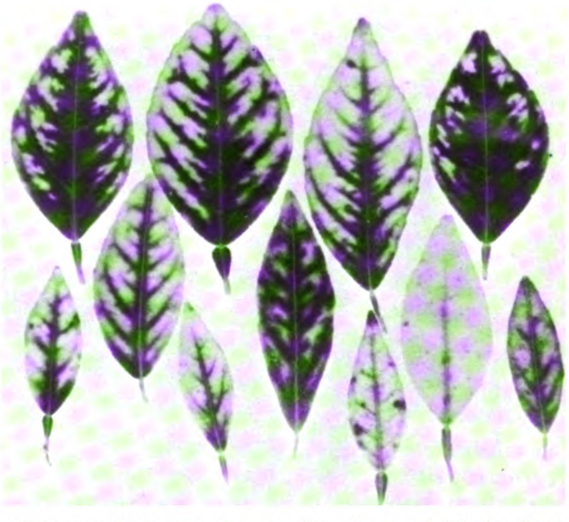

Fig. 3–66. Orange leaves showing variable leaf patterns and small, narrow leaves produced on zinc-deficient trees.

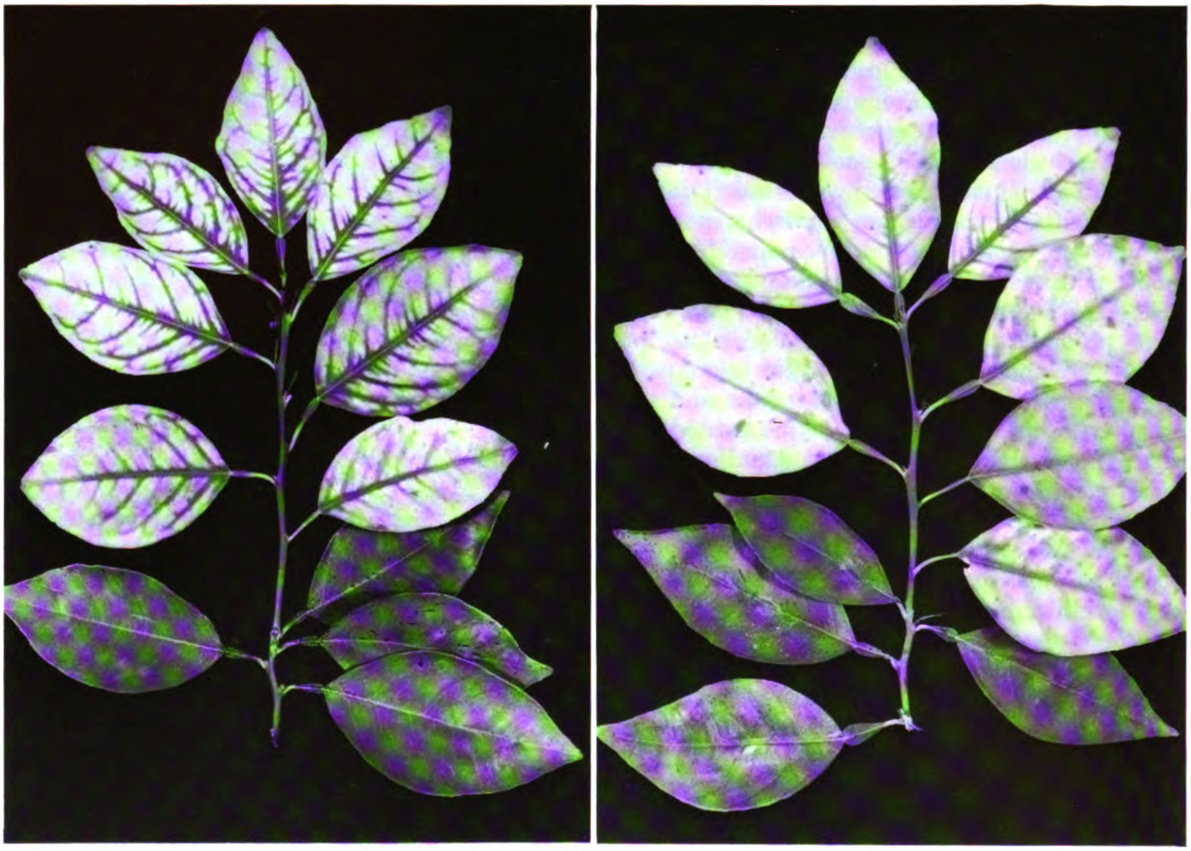

Fig. 3–67. Zinc deficiency effects on terminal fall growth of Valencia orange, showing acute stages.

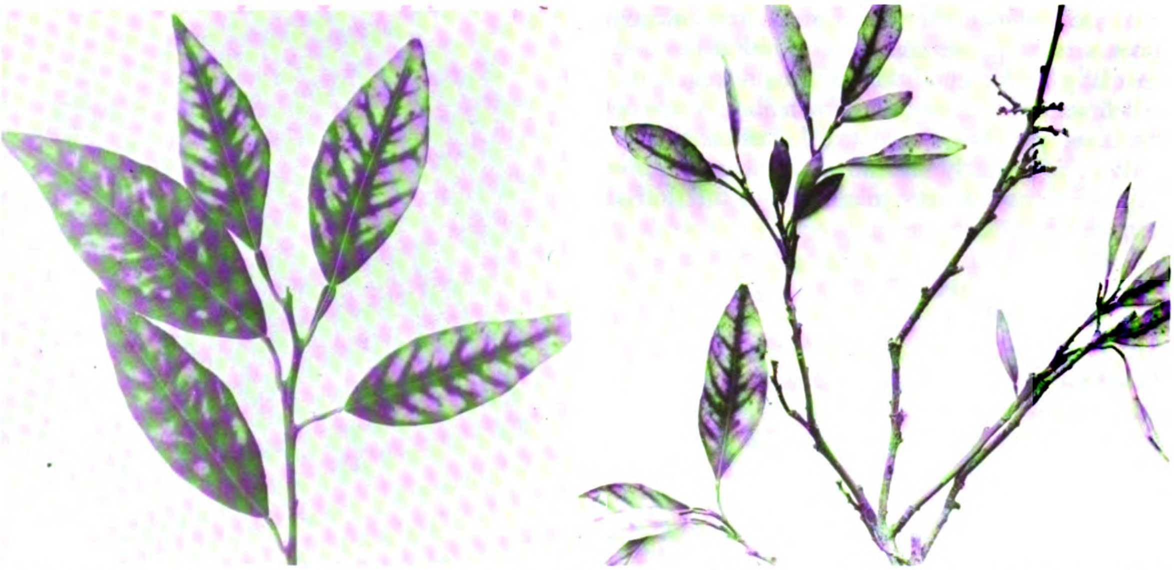

Fig. 3–68. Orange twigs showing patterns and effects of zinc deficiency. Left, moderate damage. Right, acute damage.

the persistent efforts and curiosity of research scientists.

The striking and characteristic visual symptoms produced on citrus by lack of zinc have made it one of the easiest nutritional disorders to identify and this, plus the fact that it occurs under a wide range of soil conditions, is the chief reason why zinc deficiency has been noted in every citrus-growing region of the world. Next to nitrogen, it is the most widespread of all nutritional disorders of citrus.

Symptoms of Zinc Deficiency.—The most striking effects of zinc deficiency on citrus are: (1) characteristic, irregular, and chlorotic leaf spots (thus the name "mottle-leaf"); (2) small leaves on terminal growth; and (3) severe dieback of twigs. As with manganese deficiency, the first leaf effects are a fading of chlorophyll in mesophyll areas between the veins, with the main veins, midribs, and bands or fringes adjacent to these remaining green except when the deficiency becomes very acute. Thus, a series of irregular, chlorotic blotches and patterns appear on leaves, as illustrated in figures 3–66 through 3–70. There is usually more complete fading of chlorophyll in the interveinal areas, with sharp yellow and cream colors appearing, than is the case with manganese deficiency. Also, marked reduction in leaf size and a tendency for leaves to be narrow and elongated is another characteristic. Decreased stem elongation and shortened internodes resulting in small leaf clusters produce a rosetted appearance of twigs. In addition, the mottling with zinc deficiency is more acute on the sunny side of the tree, whereas with manganese deficiency, leaf patterns are generally more pronounced on the shady side of the tree. Weakened terminal growth (due to the poor, weak, chlorotic, and small leaves) result in marked twig dieback, and trees severely deficient become open and bushy in appearance.

Another characteristic of zinc-deficient leaves is the frequent presence of small green dots in the yellowed areas. This is a further distinguishing feature not seen in manganese-deficient leaves. The small, narrow, and pointed leaves often stand more erect (the angle made between the leaf and stem is more acute than is the case with iron- and manganese-deficient leaves).

In many areas, zinc deficiency is more prevalent in the fall than in the spring, but this tendency varies some. In India, zinc deficiency commonly comes on strongly during the late spring and summer; in the fall, at the cessation of monsoon rains, there is often considerable new growth, which is normal in appearance.

Fruit production is markedly curtailed when zinc deficiency reaches an acute stage and may be a little depressed even in the early stages. This is the reason why a fertilizer program designed to avoid the first appearance of mottled leaves is desirable. In a study of grapefruit yields in relation to mottle-leaf severity, Parker (1937*b*) found little yield reduction when only a small amount of foliage was visually affected. However, Embleton, Wallihan, and Goodall (1965) obtained evidence that some yield decrease of lemons may occur even at earliest stages of deficiency, when there are no visible symptoms and only low-zinc levels in lemon leaves.

Fruit from zinc-deficient trees is small, and, if the deficiency is severe, oranges and grapefruit may be thick-skinned and misshapen. In such cases, the pulp is woody, dry, insipid, and low in acid and vitamin C. Lemon fruit is smaller, elon-

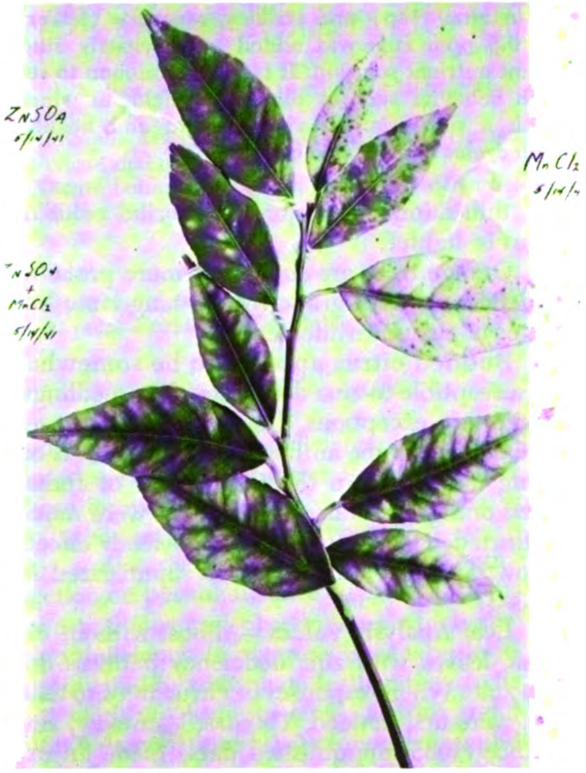

Fig. 3–69. Lemon shoot showing combined zinc and manganese deficiencies. Only partial recovery occurred from zinc and manganese leaf treatments applied separately. Complete greening occurred when a solution containing both zinc and manganese was applied to a leaf.

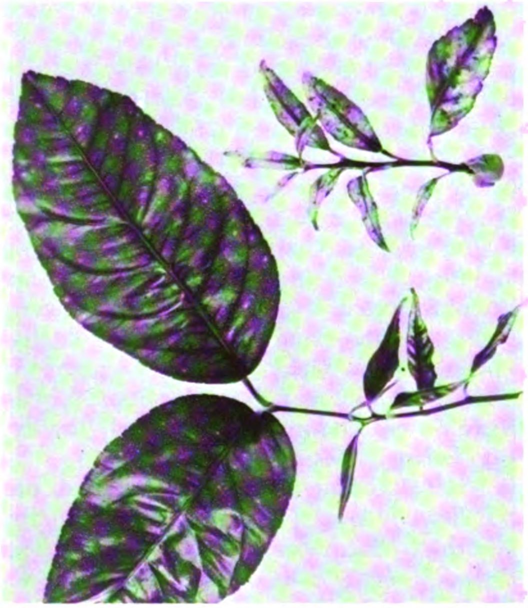

Fig. 3–70. Terminal twigs of lemon acutely deficient in zinc (top) and striking recovery (lower) when 0.5 ppm zinc was added to previously zinc-deficient nutrient solution. It is not uncommon to see growth behavior such as this in the field, in which there is a succession of zinc-deficient and healthy growth cycles on the same branch.

gated, thin-skinned, and juicy. The rind color of oranges is lighter.

Orange trees are somewhat more prone to zinc deficiency than grapefruit or tangerine, but all types of citrus are affected.

Budded citrus appears to be somewhat more susceptible to zinc deficiency than seedlings, but there are exceptions. One of the worst cases of zinc deficiency the author has ever seen was on seedling mandarins in the Coorg area of India; budded orange trees in the same soil were much less affected. The principal zinc deficiency effects on growth, foliage, and fruit are summarized in table 3–37.

Leaf Analysis Values.—Almost without exception, leaves with zinc deficiency patterns are low in zinc. When responses of citrus trees to zinc sprays were first noticed, Thomas, Vanselow, and Laurance (1935, unpublished data) made an extensive collection of leaves from mottle-leaf-affected and normal citrus orchards in California. Leaves with various degrees of mottle usually showed less than 15 ppm zinc in the dry matter, whereas green leaves were usually higher. Others have since reported analyses of leaves from zinc-deficient and nondeficient orchards (Gaddum, Camp, and Reuther, 1936; McGeorge, 1939; Healy, 1952; Sato *et al.*, 1952; Khanduja, 1955; and Kanwar and Dhingra, 1962). With few exceptions the results of these investigations confirm that leaves from zinc-deficient trees range from about 4 to 20 ppm of zinc in the dry matter, whereas leaves from healthy trees showing no signs of zinc deficiency usually contain more than 20 ppm. However, there is some overlapping, as is true with most other nutritional deficiencies, and the author knows of orchards showing 10 to 12 ppm of zinc in the dry matter of nine- to ten-month-old spring-flush leaves which show no mottle.

Zinc deficiency markedly affects some of the other inorganic constituents of leaves. Kelley and Cummins (1920) reported analyses of normal and mottled orange, lemon, and grapefruit leaves. Some of their data are reproduced in table 3–38. The data show clearly that calcium is lower and potassium, phosphorus, and nitrogen are markedly higher in mottled (zinc-deficient) leaves than in healthy, green leaves. These symptoms are usually regarded as effects of zinc deficiency rather than causes, but it is of interest that high phosphorus can also bring on or aggravate zinc deficiency. Although the evidence is less extensive, high nitrogen and potassium are thought to aggravate this disorder (Reuther and Smith, 1950).

Causes of Zinc Deficiency.—Zinc deficiency of citrus and other plants occurs on a wide range of soils. Many conditions and factors can cause this disorder. According to Swaine (1955), total zinc in soils normally ranges from < 10 to 300 ppm, but much higher levels can be found in soils contaminated by industrial sources, mine wastes, and ores.

Low total-zinc content, soil alkalinity, and high phosphorus (the latter either naturally occurring or resulting from fertilizer applications), nitrogen fertilizers (especially alkaline-forming types), organic matter, soil moisture, excess potassium, excess copper, and imbalances from other elements have all been noted as variously involved or implicated in zinc deficiency.

Zinc deficiency is especially prevalent in acid-leached, sandy-type soils where the cause is commonly low-zinc content. It also occurs widely in alkaline soils, and in these low-zinc solubility is the chief cause. Both low- and high-zinc values have been reported in various peat and muck soils. In the acid sands of Florida and other areas, liming has frequently brought on zinc deficiency

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Table 3–37

PRINCIPAL ZINC DEFICIENCY EFFECTS ON CITRUS AND OTHER FACTORS WHICH PRODUCE IDENTICAL OR SIMILAR EFFECTS

Zinc Deficiency Effects	Other Factors Producing Identical or Similar Effects
Foliage and Growth Characteristics	
1. Terminal shoot leaves show a chlorotic mottling characterized by mid-vein blotches. Chlorosis is more prominent and abundant on the sunny side of the tree.	1. Manganese deficiency produces similar leaf patterns, but the interveinal chlorosis is usually not as yellow, therefore the patterns are not as sharply defined; also the leaves on the north side of the tree are more affected, just the reverse of zinc deficiency.
2. Leaf sizes are often drastically reduced and become narrow, pointed, and chlorotic.	2. Many other conditions can reduce leaf sizes, but the dual factors of chlorosis and narrowing of leaves are peculiar to zinc deficiency.
3. Some chlorotic leaves may show small green dots; see figures 3–65, 3–66, 3–67.	3. Green dots in chlorotic areas are seen only where iron-deficient leaves have been sprayed with iron compounds.
4. Because of decreased stem growth and small leaf sizes, a rosetted condition appears.	4. Some rosetting occurs with salt excess and other conditions which limit growth.
5. Weak new growth results in twig dieback; the tree becomes defoliated and brushy-looking.	
6. Leaf chlorosis is usually more prevalent in the fall flushes of growth in California.	
Fruiting and Fruit Characteristics	
1. Yields are drastically reduced when the condition is severe. In early stages, marked by only a few visual symptoms yield may be only slightly reduced.	1. Other nutritional disorders reduce yields.
2. Fruit sizes are decreased.	2. Potassium, magnesium, and other deficiencies reduce fruit size.
3. Rinds are often lighter in color.	3. Phosphorus excess and sulfur deficiency commonly decrease rind color.
4. Fruit may be misshapen when the deficiency is acute.	4. Acute boron, phosphorus, and sulfur deficiencies produce misshapen fruit.
5. Vitamin C is decreased.	
Leaf Analysis Values	
1. Leaves showing varying degrees of chlorotic patterns will vary from 4 to 20 ppm of zinc in the dry matter; but sometimes leaves with as low as 10 ppm of zinc show the mottling.	1. Only zinc deficiency results in low zinc values, so far as present evidence is available.

(Floyd, 1917; Lott, 1939; Wear, 1956; Seatz *et al.*, 1959). Jurinak and Thorne (1955) studied zinc solubility in bentonite systems. Minimum zinc solubility in acid-bentonite suspensions treated with increasing amounts of sodium and potassium hydroxide was in the pH range of 5.5 to 7. The amounts of zinc in solution per gram of clay in this range amounts to around 1 μgm of zinc per gram of bentonite, or 1 ppm. When treated with calcium hydroxide, minimum solubility was at pH 7.5 and above, and the amount of zinc in solution was around 0.5 ppm. In systems treated with sodium and potassium hydroxide, zinc solubility increased at pH values above 7, probably because of the formation of zincate ions. Zinc solubility did not increase in the calcium systems as pH increased.

Soils derived from granites and gneiss are more prone to zinc deficiency, and, as already noted, mottle-leaf of citrus in California is much more prevalent on sandy, loamy, and gravelly soils than on heavier clay types.

Already discussed at some length in the section on phosphate excess (p. 211) are the many investigations and observations which show that zinc deficiency in some soils can be induced or aggravated by phosphate fertilization. In field and pot work not hitherto cited, Fortini and Morani (1960) demonstrated that monocalcium phosphate (at soil application rates of 330 to 1,000 ppm of P_2O_5) depressed zinc uptake by tomato and spinach on soils low in zinc. Applications of zinc sulfate at rates providing 20 ppm of zinc eliminated the phosphate-induced zinc deficiency. Many researchers are now of the opinion that the phos-

Table 3–38

INORGANIC COMPOSITION OF NORMAL AND MOTTLED LEAVES FROM VARIOUS KINDS AND VARIETIES OF CITRUS

Kind of Citrus or Variety	Condition of Leaves	Per Cent in Dry Matter of Leaves								
		SiO_2	Ca	Mg	K	Na	P	S	N	Cl
Navel orange	Normal	0.34	4.73	0.24	1.13	0.13	0.14	0.45	2.70	0.04
	Mottled	0.21	2.85	0.26	1.96	0.13	0.25	0.46	3.12	0.04
Valencia orange	Normal	0.42	4.86	0.21	0.59	0.13	0.11	0.30	2.31	0.03
	Mottled	0.25	3.01	0.22	1.62	0.09	0.25	0.30	3.21	0.09
Lemon	Normal	0.56	5.91	0.32	0.72	0.12	0.16	0.29	2.10	0.03
	Mottled	0.24	3.84	0.33	2.08	0.03	0.26	0.36	2.40	0.05
Grapefruit	Normal	0.38	5.51	0.41	1.06	0.04	0.21	0.22	1.98	0.02
	Mottled	0.28	3.91	0.41	2.46	0.05	0.32	0.33	2.86	0.05

Source: Kelley and Cummins (1920).

phorus effect is due more to reactions within the plant than solubility effects in the soil.

Nitrogen fertilization often increases the severity of zinc deficiency (Ozanne, 1955; Chapman *et al.*, 1937; Camp, 1945; Reuther and Smith, 1950; Haas, 1936*d*). This may be related more to associated soil pH changes or to cation increases in the soil solution rather than to the specific effects of nitrogen per se. For example, Viets, Boawn, and Crawford (1957) found that sodium nitrate decreased zinc uptake, whereas ammonium nitrate and ammonium sulfate increased zinc uptake. In later work, Boawn *et al.* (1960) found that uptake was not as good with calcium nitrate as with the ammonium forms. However, Wear (1956) found that substantial increases in the calcium of soils (through gypsum application) did not decrease zinc uptake.

Organic materials may either increase or decrease zinc availability. Miller and Ohlrogge (1958*a*, 1958*b*) found that aqueous extracts of ground alfalfa and manure could complex zinc and decrease zinc uptake by corn and soybean from nutrient solutions. On the other hand, it has been a common observation in California citrus orchards that winter cover crops (turned under in the spring) and organic manures usually decrease the severity of zinc deficiency.

Soil Analysis Values.—Though very little soil analysis data relating the zinc content of orchard soils to citrus performance have been developed, considerable data have accumulated with other crops. A few investigations will be mentioned here.

Brown, Krantz, and Martin (1962) found with corn growing in potted soils that 0.55 ppm of dithizone-extractable zinc was a level below which this crop responded to zinc applications and above which there was little response.

Shaw and Dean (1952) found for many crops that a value of 1 ppm of zinc, and less in soils of pH 7 and higher, indicated a need for zinc. Their extractant was a solution of ammonium acetate and dithizone-carbon tetrachloride. With soils of pH 6 and lower, 2.5 ppm of zinc was ample.

Massey (1957), working with thirty-four silt-loam soils of Kentucky, found that zinc uptake by corn in pots was related to pH and to the amount of zinc extracted by Shaw and Dean's ammonium acetate-dithizone-carbon tetrachloride reagent. He proposed the multiple regression formula:

$$\hat{Y} = 99.2 - 12.2X_1 + 10.9X_2,$$

where X_1 is pH, X_2 is the dithizone-extractable zinc in ppm of dry soil, and $\hat{Y}$ is the calculated zinc uptake expressed in micrograms of zinc. Where the value of $\hat{Y}$ is greater than 40, no zinc deficiency can be looked for; and where it is lower, the chances are good that responses to zinc will be obtained. A multiple correlation coefficient of 0.802 was found between zinc uptake and the pH and zinc analysis values.

Wear and Sommer (1948), using 0.1*N* HCl and a 1-to-10 soil-extractant ratio, found that zinc deficiency occurred in general when soil values of zinc were less than 0.90 ppm.

Other methods have been proposed but the aforementioned are sufficient to show that some indication of zinc status may be had from soil test methods. As with all availability tests, the nature of the crop, associated soil conditions, climate, soil organisms, aeration, soil moisture, and other factors impose qualifying conditions of sufficient magnitude so that soil tests provide information

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for general guidance only. Leaf analyses for zinc and visual symptoms will provide a more certain guide with citrus and many other crops. However, soil analyses are especially useful in indicating potential supplies and trends toward zinc excesses. Continued use of foliar sprays or injudicious applications of zinc salts to soils may eventually lead to iron chlorosis and other injury effects.

Control of Zinc Deficiency.—Soil applications of zinc sulfate to correct zinc deficiency of citrus fell into disrepute in the early years of experimental work in California for the reason that while correction could often be achieved by spreading zinc sulfate in a circle of 4- to 5- foot radius from the trunk, tree injury frequently occurred. In order to avoid injury and still get correction, the amounts applied had to be tailored to each soil type.

When it was found that complete correction could be obtained from foliar sprays and correction would hold for one to several years, attempts to work out soil-treatment methods were abandoned.

However, with the development of chelates, renewed research on soil application trials has begun. Some of this work merits mention and will be discussed, along with other soil application techniques, after mention of foliar sprays in the section which follows.

Foliar sprays.—A great deal of early work on the use of foliar sprays was carried out by Johnston (1933) and Parker (1934, 1936, 1937*a*, 1938), in which materials, rates, timing, lasting effect, and other matters were investigated. It was found that zinc metal dusts, zinc sulfide, zinc oxide, zinc sulfate, and other zinc-containing materials all gave some correction. Also, many formulations with copper and manganese sulfates, boric acid, sodium molybdate, urea, sulfur, lime sulfur, oil, and many other insecticides have been tried and are extensively used.

Some of the many formulations currently in use or possible in California are tabulated in table 3–39. In many cases, a single foliar application of a suitable zinc compound annually will suffice. However, where the condition is severe or where special conditions prevail, two or more applications annually may be needed. Best results are generally obtained by spraying when the spring-flush growth is one-third to two-thirds developed. Poorest results are obtained in winter months or when sprays are applied to old, hardened leaves. Timing is thus of considerable importance.

Soil applications.—As stated earlier, soil applications of zinc sulfate in California applied in a circle around the trunks of citrus trees, while frequently effecting recovery of zinc-deficient trees, fell into disrepute owing to the difficulty of adjusting the rates so as to secure control without producing severe injury. Also, the very clear evidence that slight excesses of soluble zinc (as with copper) can cause iron chlorosis is a further reason why soil applications have not been encouraged. (In time, of course, residues from foliar spray may build up sufficient zinc in soils to prove toxic, especially if acid-forming fertilizers such as ammonium sulfate are used in sufficient amounts to increase soil acidity.) In spite of this hazard, the possibility of finding suitable compounds and methods of soil applications should not be dismissed.

In a recent experiment, Embleton *et al.* (1965), working with Eureka lemons on a fine sandy loam of about pH 7.3, increased foliage zinc content by applying zinc sulfate in two successive doses at a rate providing 3.6 pounds of metallic zinc per tree, the first time in a 3-inch band around the drip of the tree and five months later in six shallow holes around the drip line. When used in combination with a foliar manganese sulfate spray, this treatment increased yields by about 125 pounds per tree over a 31-month period. Recently matured leaves, sampled at five and seventeen months, respectively, after the last application of zinc sulfate, showed zinc levels of 23.5 to 28.7 ppm of zinc in the dry matter as contrasted with 14.9 to 20.4 ppm in the control trees.

With respect to the use of zinc chelates, there is evidence that ZnEDTA and ZnHEEDTA are more effective sources of zinc for many plants and on many soils than zinc sulfate. Stewart and Leonard (1955, 1957) and Leonard, Stewart, and Edwards (1957) found that the zinc of ZnEDTA would move more readily in soils than the zinc of zinc sulfate. More zinc from ZnEDTA applications was absorbed by plants from soils of pH 6 to 7 than from soils of pH 4.5. Soluble iron readily displaces the zinc in ZnEDTA. This probably accounts for the poorer performance of ZnEDTA in acid soils.

With avocados, Wallihan, Embleton, and Printy (1958) found that 1 pound of ZnEDTA per tree increased the zinc content of leaves from 15 to 50 ppm, and the effect lasted two and one-

(Text resumes on page 242)

Table 3–39

SAMPLES OF SPRAY AND DUST FORMULATIONS USEFUL IN CORRECTING ZINC DEFICIENCY OF CITRUS IN CALIFORNIA

Correction Sought	Weight in 100 Gallons of Water: Ingredients*	Weight in 100 Gallons of Water: Element Basis	Tree Coverage	Best Time to Apply	Comments
Zinc deficiency	3 lb of $ZnSO_4 \cdot H_2O$ (36% Zn) and 1.5 lb of Na_2CO_3	1.08 lb of Zn	Skeleton	After spring bloom when new foliage is 1/3 to 2/3 expanded.	Can be applied any time of year, but best in spring, or early summer.
Zinc deficiency	0.5 lb of $ZnSO_4 \cdot H_2O$ (36% Zn)	0.18 lb of Zn	Skeleton	After spring bloom when new foliage is 1/3 to 2/3 expanded.	Can be applied any time of year, but best in spring, or early summer.
Zinc deficiency	1.25 lb ZnO (80% Zn)	1.00 lb of Zn	Skeleton	After spring bloom when new foliage is 1/3 to 2/3 expanded.	Can be applied any time of year, but best in spring, or early summer.
Zinc and manganese deficiency	3 lb of $ZnSO_4 \cdot H_2O$ (36% Zn), 3 lb of $MnSO_4 \cdot H_2O$ (32% Mn), and 3 lb of Na_2CO_3	1.08 lb of Zn and 0.96 lb of Mn	Skeleton	After spring bloom when new foliage is 1/3 to 2/3 expanded.	Can be applied any time of year, but best in spring, or early summer.
Zinc, manganese, and copper deficiency	3 lb of $ZnSO_4 \cdot H_2O$ (36% Zn), 3 lb of $MnSO_4 \cdot H_2O$ (32% Mn), 1 lb of $CuSO_4 \cdot 5H_2O$ (25% Cu), and 4 lb of $Ca(OH)_2$	1.08 lb of Zn, 0.96 lb of Mn, and 0.25 lb of Cu	Skeleton	After spring bloom when new foliage is 1/3 to 2/3 expanded.	Can be applied any time of year, but best in spring, or early summer.
Zinc and nitrogen deficiency	3 lb of $ZnSO_4 \cdot H_2O$ (36% Zn), 7.5 lb of urea (46% N; contg. <0.25% biuret), and 1.5 lb of Na_2CO_3	1.08 lb of Zn and 3.4 lb of N	Thorough coverage	After spring bloom when new foliage is 1/3 to 2/3 expanded.	Can be applied any time of year, but best in spring, or early summer.
Zinc deficiency and *Dothiorella* fungi	2 lb of Zineb (zinc ethylene bisdithiocarbamate) 15% Zn content	0.30 lb of Zn	....	....	....
Zinc deficiency, orange tortrix, Fuller rose beetle, and katydids	10% of ZnO + 90% of a 50% Cryolite dust at 90 lb per acre	About 0.08 lb of Zn and 0.45 lb of Cryolite per pound of dust	About 1.0 lb per tree	Apply when larvae appear in sufficient numbers.	....
Zinc deficiency, citrus red mite, six-spotted mite, and Yuma spider mite	0.25 lb of ZnO, and 20 lb of Aramite (15% WP)	0.20 lb of Zn	Mist spray	Apply as mites appear, except during bloom.	Use only on nonbearing trees.
Zinc deficiency, Pacific spider mite, and citrus flat mite	0.25 lb of ZnO and 1 lb of Chlorobenzilate (25% WP)	0.20 lb of Zn	Thorough coverage	Apply as mites appear except during bloom.	....
Zinc deficiency, copper deficiency, and *Septoria* fungi	3 lb of $ZnSO_4 \cdot H_2O$ (36% Zn), 1 lb of $CuSO_4 \cdot 5H_2O$ (25% Cu), and 2.5 lb of $Ca(OH)_2$	1.08 lb of Zn and 0.25 lb of Cu	Thorough coverage	In fall, when danger of *Septoria* appears.	....
Zinc deficiency, brown rot, and copper deficiency	3 lb of $ZnSO_4 \cdot H_2O$ (36% Zn), 1 lb of $CuSO_4 \cdot 5H_2O$ (25% Cu), and 2.5 lb of $Ca(OH)_2$	1.08 lb of Zn and 0.25 lb of Cu	Skirts of trees	In fall, when danger of brown rot occurs.	....
Zinc deficiency, red scale, other scales, orangeworms, citrus aphids, and katydids	0.25 lb of ZnO, 2 gal. kerosene (horticultural grade), and 1.2 lb of Parathion (25% WP)	0.20 lb of Zn	Thorough coverage	Do not apply during bloom period	....
Zinc deficiency, red scale, and other scales	0.25 lb of ZnO, 1.6 to 1.8 gal. of light or light medium petroleum oil, and 1 lb of Malathion (25% WP)	0.20 lb of Zn	Thorough coverage	August through October on oranges; April and May or September through December on lemons.	For navel oranges and grapefruit as emergency treatment only.

Zinc deficiency, red scale, other scales, citrus aphids, and citrus bud mite	0.25 lb of ZnO, 1.6 to 1.8 gal. of light or light medium petroleum oil as emulsive or 2.0 gal. as emulsion, and 0.4 lb of Parathion (25% WP)	0.20 lb of Zn	Thorough coverage	April and May or September through December.	For lemons.
Zinc deficiency, red scale, other scales, citrus mites, mealybugs, and Fuller rose beetle	0.25 lb of ZnO, 1.6 to 1.8 gal. of light or light medium petroleum oil as emulsive or 2.0 gal. of emulsion, and 0.4 lb of Parathion (25% WP)	0.20 lb of Zn	Thorough coverage	August through October.	For Valencia oranges and grapefruit as emergency treatment; *do not apply on navels.*
Zinc deficiency, citrus scales, and citrus mites	0.25 lb of ZnO, 1.6 to 1.8 gal. light medium petroleum oil as emulsive or 2.0 gal. as emulsion	0.20 lb of Zn	Thorough coverage	Late summer (August) through October on *oranges.*	For oranges and grapefruit.
Zinc deficiency, citrus scales, and citrus mites	0.25 lb of ZnO, 1.6 to 1.8 gal. of medium petroleum oil as emulsive or 2.0 gal. as emulsion	0.20 lb of Zn	Thorough coverage	Spring (April or May) and/or September through December.	For lemons.
Zinc deficiency, black scale, other scales, orangeworms, and Fuller rose beetle	0.25 lb of ZnO and 1.4 lb of DDT (50% WP)		Thorough coverage	Apply as soon as practicable following black scale hatch.	Do not apply during bloom period.
Zinc deficiency and citrus thrips	0.25 lb of ZnO and 1 lb of Dieldrin (50% WP)	0.20 lb of ZnO	Mist spray	After petal fall to prevent fruit scarring and in summer for foliage infestation.	....
Zinc deficiency, citrus red mite, and other mites	0.25 lb of ZnO and 0.8 pt of Kelthane (42% E)	0.20 lb of ZnO	Thorough coverage	Apply as mites appear.	....
Zinc deficiency, red scale, and other scales	0.25 lb of ZnO, and 2.4 to 3.4 lb of Malathion (25% WP)	0.20 lb of ZnO	Thorough coverage	Immediate postbloom period preferred.	Do not apply during bloom.
Zinc deficiency, red scale, and other scales	0.25 lb of ZnO, and 1.4 to 2.4 lb of Parathion (25% WP)	0.20 lb of ZnO	Thorough coverage	Post-bloom.	Do not use during bloom.
Zinc deficiency, red scale, other scale, and orangeworms	0.25 lb of ZnO, 1.0 to 1.2 lb of Sevin, carbaryl (80% WP)	0.20 lb of ZnO	Thorough coverage	Immediate post-bloom.	Do not use during bloom.
Zinc deficiency, citrus aphids, and citrus red mite	0.25 lb of ZnO and 0.5 pt of Systox (2 lb per gal. of E)	0.20 lb of Zn	Thorough coverage	As required.	....
Zinc deficiency, citrus red mite, Pacific spider mite, and two-spotted mite	0.25 lb of ZnO and 1 lb of Tedion (25% WP)	0.20 lb of Zn	Thorough coverage	As mites appear.	....
Zinc deficiency and citrus aphids	0.25 lb of ZnO and 1 pt of Rotenone extract (2½% of Rotenone)	0.20 lb of Zn	Outside coverage	As required, but not during bloom.	....
Zinc deficiency, citrus red mite, and six-spotted mite	0.25 lb of ZnO and 1.6 lb of Trithion (25% WP)	0.20 lb of Zn	Thorough coverage	As mites appear.	....
Zinc deficiency and preharvest fruit drop	0.25 lb of ZnO and 8 ppm of 2,4-D	0.20 lb of Zn	Skeleton	Fall and winter.	....
Zinc deficiency and European brown snail	0.25 lb of ZnO and 1.6 lb of Guthion (25% WP)	0.20 lb of Zn	Skirt, trunk, and ground litter	As needed except during bloom.	....
Zinc deficiency, red scale, other scales, and orangeworms	0.25 lb of ZnO, 1 lb of Parathion (25% WP), and 1.6 lb of Malathion (25% WP)	0.20 lb of Zn	Thorough coverage	All year except during bloom.	....

• WP = wettable powder; E = emulsifiable concentrate.

half years, but in subsequent experiments by Wallihan and Embleton, reported by Embleton and Jones (1964), not as good results were obtained.

In comparison of various zinc carriers—ZnEDTA, zinc oxide, zinc phosphate, zinc carbonate, zinc frits, zinc granules, and two smelter byproducts, applied respectively to provide 2 ppm of zinc to a fine sandy loam in pots—Boawn, Viets, and Crawford (1957) found that zinc uptake was greatest from the chelate. Except for the frits (from which no uptake occurred) and blast furnace slag (from which only a little zinc absorption occurred), the others were all about equal as zinc sources.

Wallace and Mueller (1959) found that ZnHEEDTA was a better source of zinc for soybeans growing on calcareous soils than zinc sulfate, but not as good as the latter on an acid soil.

Benson, Batjer, and Chmelir (1957) found that spring applications of 1 and 2 pounds of ZnEDTA or ZnHEEDTA, applied under the spread of the branches on sandy loam soils of pH 5.6 to 9, were effective in supplying zinc to peach and cherry trees but not to apples.

Leonard, Stewart, and Edwards (1959) and Stewart and Leonard (1963) found that a mixture of 5 pounds of zinc sulfate and 5 pounds of calcium chloride, applied in ten piles (1-foot square) around citrus trees in acid sandy soils, markedly increased the zinc uptake by the tree. Embleton *et al.* (1964) tried out this technique on several citrus orchards in California. A treatment consisting of a mixture of 2 pounds of zinc sulfate (36 per cent zinc) plus 2 pounds of calcium chloride, applied in five piles around the dripline of the tree on two-year-old Valencia oranges (on Troyer citrange root), increased the zinc content of leaves from 19 to 441 ppm in the dry matter. The soil was a calcareous fine sand of pH 7.5 (paste), and the orchard was irrigated by a flooding system at frequent intervals. The zinc level in the leaves from these trees was above that of the controls for two years, and, while there was some initial leaf burn, no permanent injury resulted. Zinc sulfate alone applied to the soil, at the same rate, also produced some initial but lower zinc increases in foliage, but the effect did not last as long.

In comparable experiments with mature Redblush grapefruit trees on the same type soil, 5- and 10-pound applications of the zinc sulfate-calcium chloride mixtures increased the zinc in leaves to values up to 327 ppm. The effect was measurable for two years, but the zinc levels decreased to much lower amounts in time. The 10-pound application rate produced some leaf injury and increased the chloride content of the leaves a little, but not enough to prove serious.

On a heavier-type soil (Yolo sandy loam of pH 8), trials with 2½, 5, and 10 pounds of the zinc sulfate-calcium chloride mixture, applied in six holes on two furrow sides of lemon trees, did not increase the zinc content of leaves nor produce injury. Zinc sulfate alone at 20 pounds per tree did increase zinc levels in the trees.

From this brief review, it is evident that with some soils and plants zinc chelates at rates of ½ to 2 pounds of ZnEDTA or ZnHEEDTA per tree will correct zinc deficiency. Results with the zinc sulfate-calcium chloride mixtures have been successful in some cases and not in others. Costs and possible toxicity suggest that soil applications are still not practical. Foliar sprays remain the safest and cheapest means of correcting zinc deficiency of citrus trees.

Zinc Excess

Leaf burn, defoliation, and twig dieback have been noted when too much zinc sulfate is applied to soil. As previously stated, because of the difficulty of determining both effective and safe rates under different soil, climate, and tree conditions, soil applications of soluble forms have been largely discontinued.

Foliar sprays formulated from soluble zinc compounds, if used at rates not exceeding 1 pound of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) per 100 gallons of water, are not apt to produce leaf burn. In greater amounts, a precipitating agent (either sodium carbonate or lime hydrate) is needed.

Excess zinc in the nutrient medium below the levels which cause severe root and top injury will often produce iron chlorosis. This was first noted by Chapman *et al.* (1940) in a series of nutritional experiments. Similar results have been obtained by Smith and Specht (1953) and with other crops by Hewitt (1948), and Hunter and Vergnano (1953), Millikan (1947*b*), and others.

Insufficient data exist for a certain diagnosis of zinc excess in citrus, but leaf analyses values of 100 ppm of zinc in the dry matter and over, especially if accompanied by iron chlorosis, would be suggestive. Soil analysis values well above those mentioned as critical for zinc deficiency would also be indicative. More information on the subject is needed.

Control of excess zinc in soils could probably be easily achieved by applications of lime and/or superphosphate.

SALINITY AND ALKALI

The sensitivity of citrus trees to excess soluble salts (salinity, alkali) and sodic salts and the effects thereof soon became apparent to early California workers. Enough information now exists to diagnose those cases where serious injury occurs from total salt or from specific ions such as sodium, chloride, sulfate, carbonate, bicarbonate, boron, and lithium.[4] However, much still remains to be learned about the influence of intermediate salinity levels as they affect citrus performance, the influence of variable nutritional factors on ion uptake, tissue and soil analyses criteria, rootstock and scion effects, moisture, climate, disease, and insect interrelations.

The effects of boron, lithium, and sulfate excesses have been discussed in previous sections. The information available on chloride, sodium, and total salt will be dealt with in this section.

Saline Conditions.—At the turn of the century, Loughridge (1900) investigated certain orange orchards near Corona, California. These orchards had been severely injured by the use of saline irrigation water from Lake Elsinore. Analysis showed that the water of Lake Elsinore at that time contained 917 ppm of sodium chloride, 377 ppm of sodium sulfate, and 391 ppm of sodium carbonate and bicarbonate considered jointly. Loughridge concluded that the injury was caused mainly by the carbonates. A little later, when attention began to be focused on chloride in relation to citrus trees, Hilgard (1900) concluded that sodium chloride was the constituent of the Lake Elsinore water mainly responsible for injury to these orange trees.

Subsequently, Loughridge (1901) directed attention to the extreme sensitiveness of the lemon tree to salt. He said: "The lemon seems to be the least tolerant of all the fruit trees, for it was stunted by 1,440 pounds common salt (sodium chloride) per acre, distributed through 4 feet depth, and was killed by 1,900 pounds of sodium carbonate." This would amount to 90 ppm of sodium chloride in a dry soil basis and about 120 ppm of sodium carbonate.

Supporting the conclusions of Loughridge and Hilgard, Kelley and Thomas (1920) showed that various species of *Citrus* are extremely sensitive to chlorides. Haas (1932*b*) showed that chlorine tends to accumulate in the leaves of citrus trees, and that the trees, when grown in culture media containing relatively high concentrations of chloride, absorb large quantities of this element. He found that under such conditions the leaves became severely burned; moreover, an analysis showed that the injured leaves contained abnormally large amounts of chlorine.

Since common salt (sodium chloride) is a constituent of many of the soils and irrigation supplies available in various semiarid regions where citrus is grown, the sensitivity of citrus trees to chloride is a matter of importance. For example, Kelley and Thomas (1920) showed many citrus orchards, situated in several different counties in California, had been severely injured by the salts (chiefly chlorides) from irrigation waters.

In discussing this problem, both Loughridge (1900) and Kelley and Thomas (1920) emphasized the fact that the injurious effects of saline irrigation water may vary greatly in different localities, and even in different parts of the same orchard. This variability seems to be caused by differences in soil character. Other conditions being similar, it is reasonably certain that the effects of a given supply of saline irrigation water will be determined by its effects on the concentration of the soil solution, on the one hand, and on soil permeability on the other.

Figure 3–71 shows an orange orchard in the area of Riverside, California, completely defoliated by saline irrigation water high in chloride. When the photograph was taken, this grove had been irrigated for about sixteen years with water containing 500 ppm of chlorine. For several years before better water was obtained, these trees continued to show pronounced chloride injury; the smaller twigs died back each year, and some defoliation took place several different times. The toxicity became apparent only after the orchard had been irrigated with saline water for several years. Although the injury became severe and trees almost ceased to bear, they were not killed by the salts of the irrigation water. After about twenty years of regular application, the saline irrigation water was replaced by nonsaline irrigation water. The result was remarkable. With-

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[4] A good compilation by D. L. Carter entitled *A Bibliography of Publications in the Field of Saline and Sodic Soils (Through 1961)* was published in 1962 by the U.S. Department of Agriculture, Agricultural Research Service (Carter, 1962).

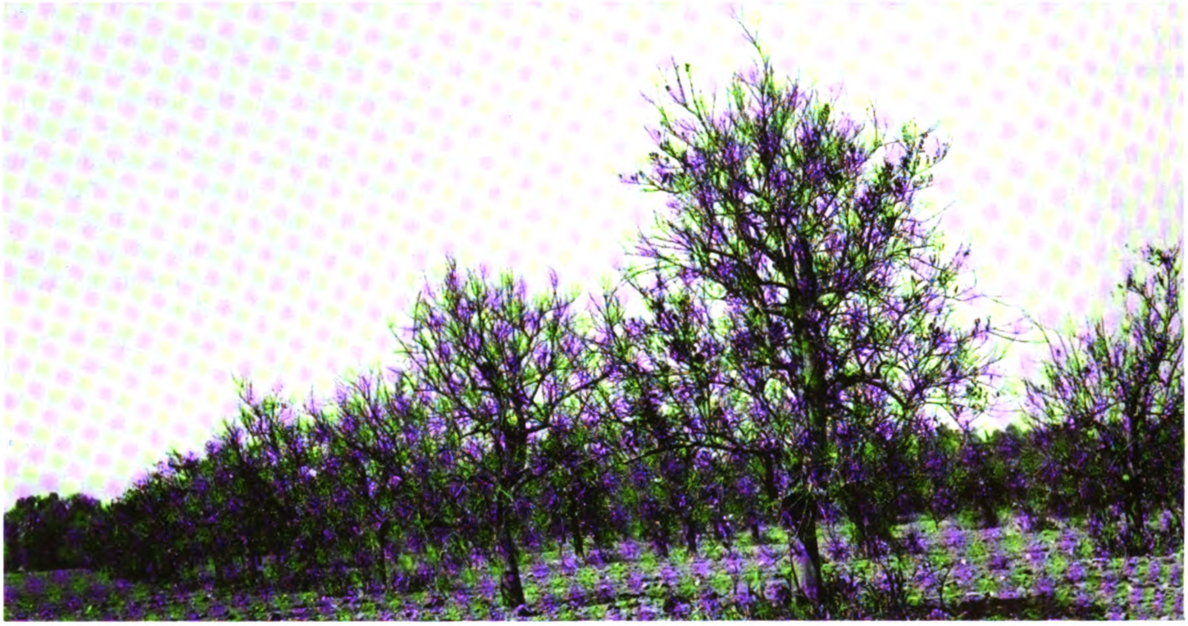

Fig. 3–71. Orange orchard showing complete defoliation resulting from excessive chloride accumulation in the leaves. This photograph was taken in the fall of the year, when salt injury is most severe. The orchard had been irrigated with water containing 500 ppm of chlorine for sixteen years before this photograph was taken in 1923.

in a year or two, the trees began to recover, and after a few years their recovery became essentially complete (fig. 3–72), though the trees never attained full size.

Thus, it is evident that injury to citrus trees produced by saline irrigation water is not necessarily permanent, though more or less permanent stunting may result, especially when young trees are injured. In fact, there are many citrus orchards in different citrus districts of California in which recovery from severe salt injury has been effected simply through applying nonsaline irrigation water by the usual method of furrow irrigation. Studies on the soils of these orchards showed that the soluble salts which had accumulated in the soil from applying saline water were gradually leached out by the combined effects of rain and nonsaline irrigation water. Conditions favorable for the growth of citrus trees were restored. Under California desert to semidesert conditions, it is not usually safe to irrigate citrus trees regularly with water containing more than 150 to 200 ppm of chlorine.

Kelley and Thomas (1920) found that in certain localities in California the regular use of irrigation water containing as little as 200 ppm of chlorine for a period of from fifteen to twenty years resulted in a slight injury to lemon trees. In these orchards, they found that the chloride content of the soil had been materially increased. The question naturally arises: what is the upper limit of chloride tolerance for citrus trees? At present, no categorical answer can be given to this question. In the first place, the effect of chloride and other soluble materials will depend upon the concentration of constituents in the soil solution. It has been established that the application of a given amount of soluble salt may produce materially different effects on the concentrations of the soil solutions of different types of soil, depending on water-holding capacity, porosity of the soil, and the rate of evapotranspiration. The effect of a given soluble constituent also is materially influenced by the amounts of other constituents present in the soil, especially the content of organic matter and nitrogen. In addition, climatic conditions influence the results, and, finally, rootstock and variety are important.

In several California localities the presence in the soil of considerably less than 500 ppm of chlorine, expressed on the basis of dry soil, has been associated with definite injury to citrus trees. The soils in question were sandy loams.

Effects of Saline-Alkali Conditions.—Alkali

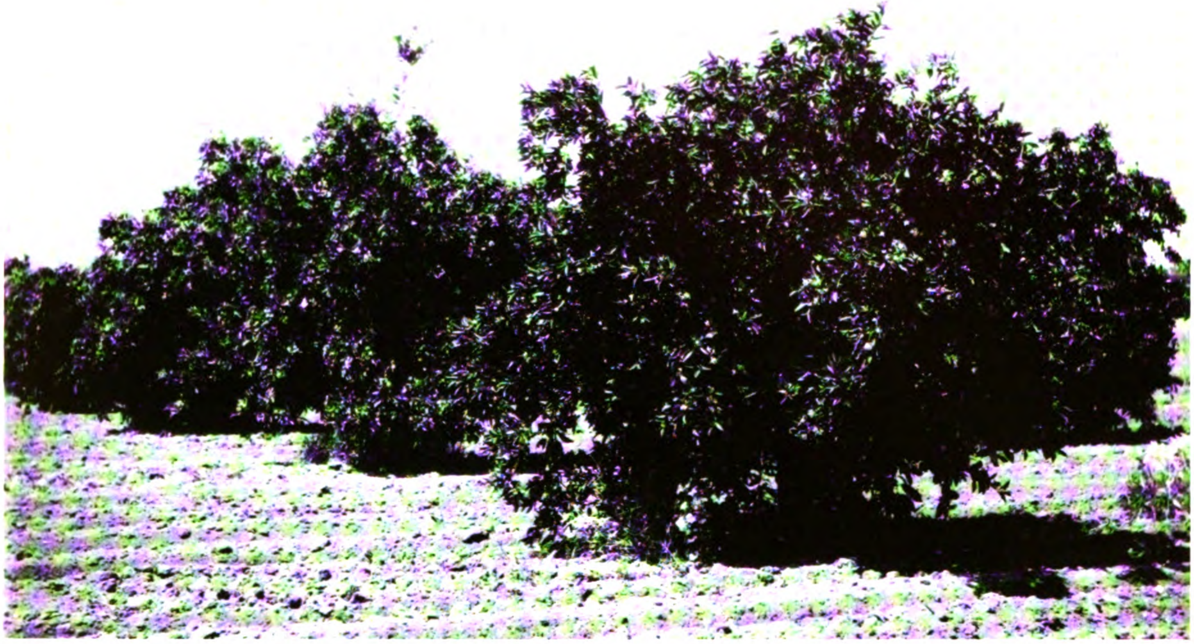

Fig. 3–72. The same orchard pictured in figure 3–71, showing recovery of the trees from chloride injury. The recovery was effected by applying nonsaline irrigation water. (Photographed in 1935.)

(high sodium) salts affect different varieties and species of *Citrus* somewhat differently. Lemon trees show the effects by pronounced yellowing and burning of the margins and tips of leaves, often followed by unusually heavy leaf drop late in the dormant season; leaves also tend to curl somewhat. Subsequent new growth may be vigorous and apparently normal for several months, but later the leaves of new shoots also turn yellow in irregularly-shaped areas along their margins and drop off excessively. Where soil contains a high chloride concentration, complete defoliation may occur, and in extreme cases death of the trees may result. The tendency toward mottle-leaf (zinc deficiency) and iron chlorosis is accentuated by moderately high concentrations of soluble salts. Both the quality and quantity of the fruit produced may be impaired. The size of the fruit will tend to be subnormal, and lemon fruits thus affected tend to become tree ripe before normal picking time.

Orange and grapefruit trees also show the effects of alkali salts in different ways. On certain soils, mottle-leaf is one of the first symptoms of injury. In the hot interior valleys of California, a moderately high concentration of soluble salts in the soil may cause older leaves to develop a brownish hue and curl to some degree. If soil contains excessive amounts of chloride, the margins and tips of leaves turn brown, and leaves may drop suddenly, especially if the soil is allowed to become unusually dry. Orange trees, thus defoliated, may later develop a profuse growth of new shoots, but new leaves are likely to be subnormal in size and pale in color.

Citrus trees injured by alkali salts are unusually susceptible to adverse climatic conditions. Hot winds cause the young leaves of such trees to dry up and fall off; frosts likewise may be unusually severe on affected trees. The relations between alkali injury and the severity of adverse climatic conditions are complex. Among the various factors, it is probable that soluble salts affect the moisture relationships of growing tissues. It has long been thought that increasing the concentration of the nutrient solution tends to cause a decrease in absorption of water by plants. Another possible reason for the extreme susceptibility of alkali-injured trees to adverse climatic conditions is their generally weakened condition.

Figures 3–73 and 3–74 show leaf-tip burning and yellowing caused by an excessive accumulation of chloride. Such leaves usually show from 0.75 to over 1.5 per cent of chlorine in the dry matter. The earliest symptom is a yellowing

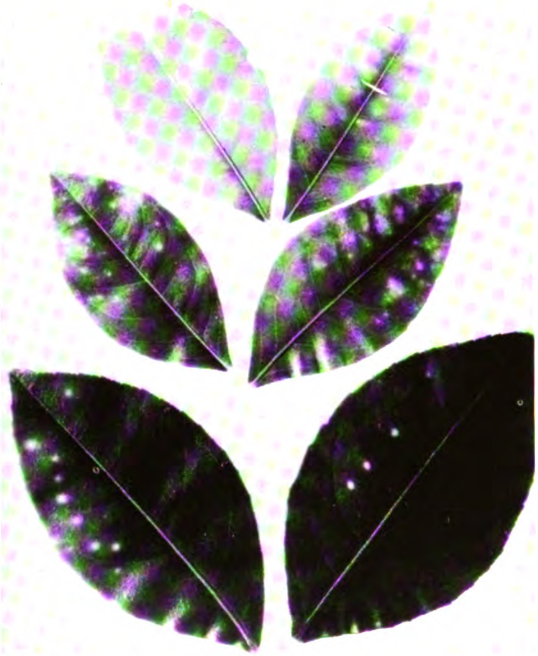

Fig. 3–73. Orange leaves showing tip burn, yellowing, and small, white spots resulting from chlorine accumulation. White spots similar to these are also caused (under some conditions) by boron accumulations.

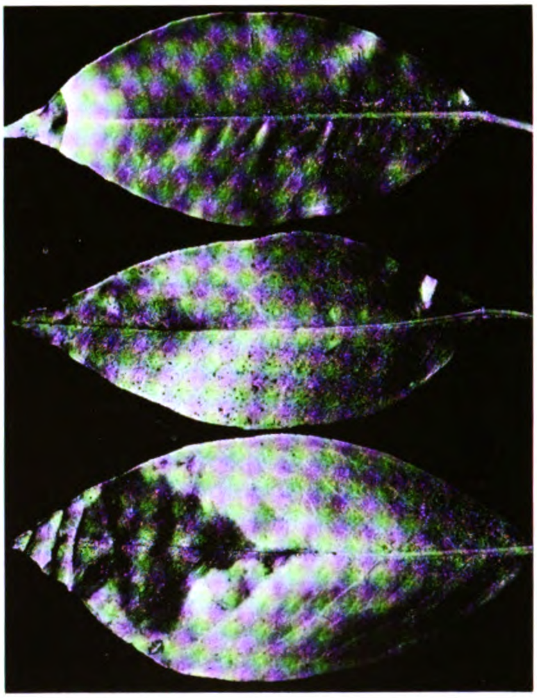

Fig. 3–74. Old orange leaves showing tip burn and yellowing or bronzing from excessive chloride accumulation.

of the tip, followed by progressive yellowing and necrosis starting at the tip and proceeding downward. Figure 3–75 shows typical necrotic burned areas on leaves caused by excessive sodium uptake.

In carefully controlled sand cultures in the greenhouse, using sweet orange seedlings, maintained concentrations of 700 ppm of chloride (supplied by increasing calcium, magnesium, and potassium levels) produced leaf symptoms of chloride excess *only* where nitrogen (as nitrate) was deficient (Chapman and Liebig, 1940). The chloride content of injured leaves in the nitrogen-deficient cultures attained values of 2.78 and 3.90 per cent in the dry matter, whereas in ample nitrogen cultures (no visual symptoms of excess chloride) the value was 0.53 per cent of chloride.

Eaton (1942) grew rooted Eureka lemon cuttings for seven months in outdoor sand cultures receiving, respectively, 0.6, 50, and 150 meq/l of chloride (50 per cent as sodium and the remainder as calcium and magnesium). Plants grown in the cultures containing 150 meq/1 chloride (5,250 ppm) became yellow; the leaves fell, and the plants died. At the 50-meq/l-level (1,750 ppm), growth was only 28 per cent of that in the low-chloride cultures. No leaf analyses were made. Seven other plants were grown under identical conditions in the sand beds. Lemon cuttings were the most seriously affected by the chloride; the order of relative growth, compared with the control as 100, was lemon, 28; navy beans, 39; dwarf milo, 54; Chilean alfalfa, 73; Acala cotton, 75; Stone tomato, 78; and sugar beet (roots), 98.

In the same publication, Eaton (1942) gives an analysis of the soil solution from two 13-year-old grapefruit orchards irrigated with Colorado River water, but growing on two different soils—one a sand, the other a silty clay. The latter orchard (fig. 3–76), while of good appearance and well foliated, produced much less fruit and the trees were smaller than those of the orchard growing on sand (fig. 3–77). Soil solution analyses from these two orchards are presented in table 3–40. Though all of the difference in growth cannot be ascribed to the difference in salt, it seems certain that the high conductivity, sodium, and chloride values found in all horizons of the clay soil were

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important as regards the difference in tree size and yields.

Hayward and Blair (1942) grew orange seedlings in nutrient solutions in the greenhouse for two months in Hoagland's solution with and without additions of 50 and 100 meq/l of mixed chlorides. Growth was reduced by 73 and 47 per cent of the control at the 50 and 100 meq/l of chloride levels. Nearly half of the plants at high-chloride levels died, and there was leaf-tip burn in the plants within two to three weeks.

Pearson, Goss, and Hayward (1957) grew Ruby Red grapefruit on Cleopatra mandarin rootstock in outdoor lysimeters filled with a loam soil. Trees were irrigated with (1) tap water of low salt content (300 ppm), (2) tap water plus 2,000 ppm of calcium chloride and sodium chloride, and (3) tap water plus 4,000 ppm of calcium chloride and sodium chloride. Ammonium nitrate at a constant rate of 0.4 gm/l was added to all irrigation waters. The trees were grown for 19 months. The average electrical conductivity (EC) of the saturation extract of soil to a depth of 0 to 48 inches for the three waters was 1.5, 6.2, and 9 mmho/cm, where free drainage of the lysimeters had been provided. Growth (fresh weight) resulting from the salt treatment, compared to controls at 100, was 46 and 38 for the two salt levels. The chloride content of four- to six-month-old leaves for the three treatments was, respectively, 0.07, 1.28, and 1.35 per cent in the dry matter.

The salt treatments resulted in a bronzing and burning of leaves. Also involved in the experiment were water-table variables, and the chief effect of high water tables was to increase salt content of the soils and severity of tree injury. A conductivity value of approximately 4 mmho/cm in the saturation extract of the soils was associated with a 50 per cent decrease in tree trunk area.

Because of a reportedly high-salt tolerance of Siamese pummelos, Monselise (1961) grew seedlings of this species in plastic buckets containing a clay soil to which enough sodium chloride had been added to produce a salinity in soil extracts of approximately EC 3.1, 5.7, and 13.5 mmho/cm. The control soil showed values of 2.55 mmho/cm. The plants were grown for about ten months. Growth was virtually unaffected, as compared to controls at the 3.1 mmho/cm salt level; at the highest level (13.5 mmho/cm), the plants died in a few months. At the 5.7 mmho/cm level, total dry weight at the end of the experiment was 28.3 per cent of the control. These results are quite consistent with those reported by Pearson *et al.* (1957). Tissue analyses of leaves and roots were made but not reported. Monselise states that sodium levels in two-month-old leaves were not in excess of what is considered normal, but that root levels were above 0.30 per cent of sodium in the dry matter.

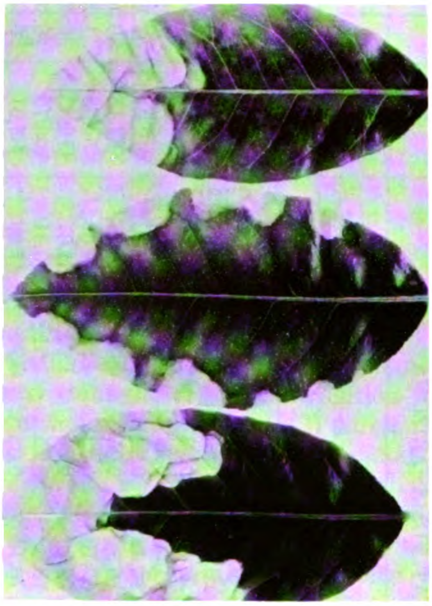

Fig. 3–75. Lemon leaves showing marginal yellowing, followed by progressive necrosis, as a result of excessive sodium accumulation. The advancing spread of the sodium burn imparts a rippled appearance in the burned areas. Sodium-injured trees, especially where heavy doses of sodium chloride or sodium nitrate have been given, may lose many leaves rather suddenly without showing any leaf burn or discoloration. The leaf burn shown in this photograph has been noted only occasionally in the field by the author. However, such symptoms have been produced repeatedly in controlled cultures from excesses of sodium from chloride, nitrate, and sulfate sources.

Chloride Accumulation and Movement.— There is much evidence that chloride in citrus (like lithium and boron) tends to accumulate primarily in leaves and this accumulation is a function of nutrient concentrations, time, and age.

Groenewegen, Bouma, and Gates (1959) exposed Washington navel orange cuttings, grown first for 74 days in a base nutrient solution, to 200 meq/l of chloride for a three-day period, having approached this high level by increasing the salt gradually (25 meq/l of chlorine per day) for some eight days. After the three-day exposure to high

Table 3–40

ANALYSES OF DISPLACED SOIL SOLUTIONS FROM GRAPEFRUIT GROVES IN ARIZONA AND CALIFORNIA AND OF IRRIGATION WATER FROM THE COLORADO RIVER AT YUMA, ARIZONA

Origin of Sample	Depth (Feet)	Moisture Equivalent	pH	EC of Soil Solution (Mmho/Cm)	Boron (Ppm)	Other Constituents in Soil Solution (Meq/L)							
						Ca	Mg	Na	K	HCO_3	SO_4	Cl	NO_3
Superstition sand	1	11.4	7.4	2.19	0.84	6.48	6.58	9.51	0.72	5.40	7.65	9.51	0.72
	2	3.4	6.4	2.26	0.44	8.71	4.47	9.62	0.80	3.89	9.62	8.66	0.98
	3	4.6	6.7	2.00	0.38	7.37	3.83	8.83	0.60	3.25	9.11	7.24	0.87
	4	5.4	7.0	2.10	0.36	7.75	4.20	9.42	0.53	2.10	11.27	7.25	0.90
	5	3.8	6.6	2.40	0.59	8.18	4.73	11.06	0.66	2.29	14.24	7.62	1.19
	6	3.9	6.6	3.20	0.48	11.79	6.62	14.49	0.92	1.75	19.43	12.21	1.22
Holtville silty clay	1	22.7	7.7	6.85	1.05	24.46	21.57	38.31	0.95	4.07	54.37	27.15	0.54
	2	18.1	7.7	10.87	1.03	37.40	28.57	69.10	0.85	4.26	75.69	54.03	1.09
	3	25.9	7.4	14.80	0.75	38.59	39.86	99.70	0.22	1.99	54.37	119.32	0.45
	4	45.7	7.3	12.29	0.87	33.39	34.07	78.92	0.15	1.51	39.81	106.75	0.40
	5	45.3	7.0	10.92	1.25	26.33	28.37	76.18	0.08	0.90	47.96	85.21	0.45
	6	37.4	7.6	7.69	0.79	18.69	18.14	54.87	Tr.	0.85	48.68	45.65	0.37
Colorado River water*		......	....	1.37	0.17	5.41	3.01	6.47	......	3.19	7.71	3.68	0.11

Source: Eaton (1942).

* Eight-year, unweighted, weekly average (Oct. 1, 1928, to Sept. 30, 1936).

chloride, salt was flushed out and plants were then harvested at progressive periods of 0, 5, 25, and 82 days following chloride removal. The investigators found very little movement of accumulated chloride out of the old leaves to younger leaves. The chief movement was from accumulated chloride in roots and stems to the younger growth. This is probably the reason there is premature abscission of salt-affected older leaves of citrus in the field, even though new growth continues to emerge. Of the total chloride found in plants at harvest (82 days after salt removal), about two-thirds was in the leaf laminae, and most of the rest was in roots and stems. At high-chloride levels, there was leaf burn and marked defoliation.

Cassidy (1946), reporting on variable salt (presumably sodium chloride) treatments to citrus trees, noted that chloride is more readily absorbed into leaves than sodium, and that the earliest symptom is a tip yellowing followed by tip burn. A dose of two hundredweight (cwt) salt per tree caused the tree to drop all leaves. The author has noted similar drastic and rapid injury and leaf drop from the addition of very high sodium chloride applications to citrus trees in the field.

Brusca and Haas (1958) grew Lisbon lemon on sour orange rootstock in large outdoor soil cultures which received complete nutrient solution additions periodically, to which were added variable amounts of chloride derived equally from calcium, magnesium, and potassium chloride. Analysis of leaves and various other parts for chlorine were made, and part of the data is presented at the top of page 249.

Very little data for citrus exist on the effects of other nutritional factors on chloride absorption, but from work with other plants there is some evidence that more chloride is absorbed when calcium is the dominant ion than when it is sodium (Elgabaly and Wiklander, 1961; Elgabaly, 1962; Brown, Wadleigh, and Hayward, 1953). It is not known whether this applies to citrus.

Sodium in Relation to Performance.—There is little evidence that sodium at any concentration or under any condition is beneficial to citrus. With some other plants or groups of plants, sodium is either essential or beneficial under varying circumstances; for example, when potassium is low. In a number of nutritional experiments, the author and his colleagues tried to find out whether sodium in either low or fairly high concentrations would partially replace or substitute for potassi-

Nutrient Solution	*Per Cent Chlorine in Dry Matter* Mature Leaves	Bark Above Bud Union	Bark of Main Root	Fine Rootlets	Blossoms	Peel	Pulp
Control	0.016	0.013	0.020	0.221	0.007	0.015	0.015
Control plus 560 ppm of chlorine	0.188	0.252	0.142	0.387	0.038	0.243	0.026
Control plus 1,400 ppm of chlorine	1.613	0.413	0.267	0.443	0.169	0.466	0.188

um. The results were consistently negative. In controlled sand cultures with orange and grapefruit seedlings and lemon cuttings, Chapman and Brown (1943) found that high-sodium levels somewhat delayed the onset of potassium deficiency but did not prevent it. Ultimately these plants (potassium-deficient) developed the same deficiency symptoms as those grown without sodium. The delaying effect of sodium was thought to be due to its effect on calcium; where this latter nutrient was high (under potassium deficiency), the plants showed acute potassium deficiency earlier than where calcium and magnesium were lower. It appears, then, that high sodium merely offsets somewhat the effect of calcium and magnesium in hastening the onset of potassium deficiency.

In studies by Pearson (1951), to be detailed later in this section, no benefits from sodium under low-potassium conditions were noted. Page and Martin (1964) explored this problem and, in soils of low or deficient potash levels, found no evidence of a beneficial effect from increasing sodium additions.

Sodium can be detrimental to citrus (and all plants) through: (1) its contribution to total salinity of soil (osmotic effect); (2) differential absorption and accumulation in roots and leaves (specific ion effect); (3) nutritional imbalancing effect (i.e., effect on absorption of other nutrients); (4) detrimental effect on soil structure and pH; and (5) leaf absorption from foliar sprays, etc. In many cases, the first four effects are combined so that it is difficult to evaluate or separate them.

This subsection will cite some specific cases in which sodium (due to one influence or another) has exerted a detrimental effect on citrus, describe leaf-injury symptoms due to excessive sodium absorption, and mention leaf values associated with excessive accumulation. In a later section on rootstocks in relation to salt injury, reference to both sodium and chloride will be found. Foliar absorp-

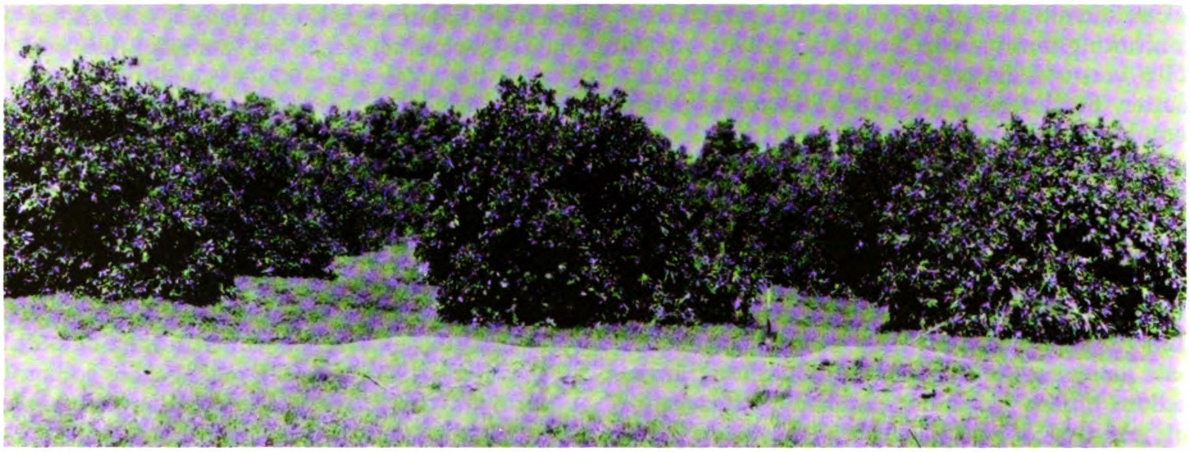

Fig. 3–76. Thirteen-year-old grapefruit grove on Holtville silty clay, Meloland, Imperial Valley, California, irrigated with Colorado River water. The yield was less than 100 pounds of fruit per tree. The character of the soil in this orchard is such that it apparently has not been possible to leach the salt residues of the irrigation water beyond the root zone. The trees have good color and are heavily foliated. There are few yellow or burned leaves. Trees and individual leaves are smaller than those of the trees shown in figure 3–77, but salt-injury symptoms of diagnostic value are otherwise lacking. Analyses of displaced soil solutions and of irrigation water are shown in table 3–40.

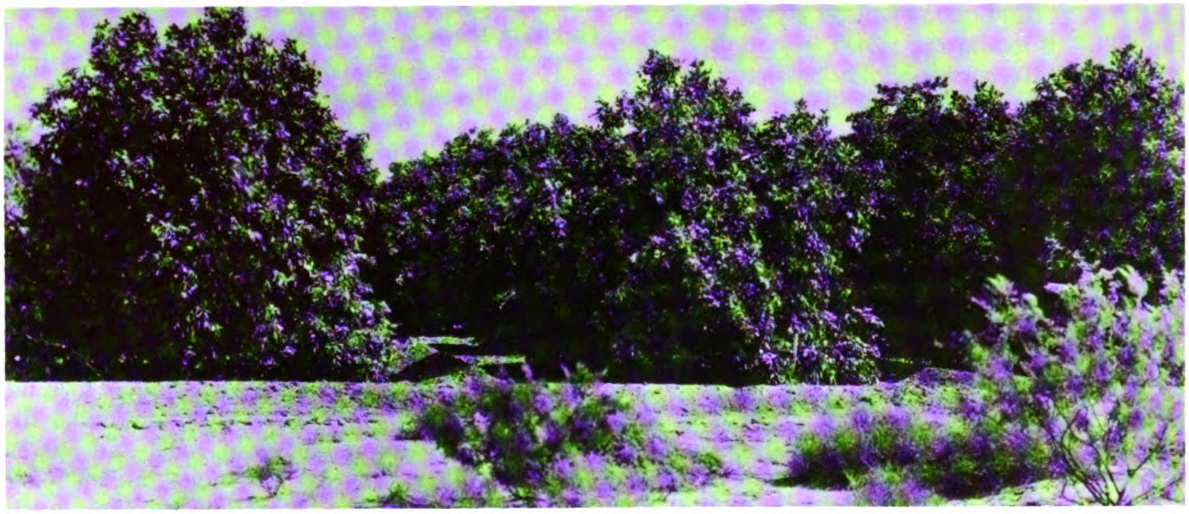

Fig. 3–77. Thirteen-year-old grapefruit grove on Superstition sand, Yuma Mesa, Arizona, irrigated with Colorado River water. The yield in 1936 was 546 pounds of fruit per tree. The character of this soil and the irrigation practices have been such that there has been little accumulation of salt in the root zone, i.e., the water has been substantially in excess of evaporation and transpiration losses. The orchard is vigorous and trees are large. Analyses of displaced soil solutions and of irrigation water are shown in table 3–40.

tion and injury from irrigation water and briny sprays will be dealt with in another section.

In a lengthy fertilizer experiment with Washington navel orange trees on sweet orange root, Jones *et al.* (1952) reported a 25 per cent reduction in yield between 1940 and 1949 from the use of sodium nitrate (at 3 pounds of nitrogen per tree per year), compared with equivalent amounts of calcium nitrate. Spring-flush leaves, picked from nonfruiting terminals in December, 1959, showed 0.106 versus 0.204 per cent of sodium in the dry matter. Feeder roots showed 0.232 versus 0.666; root bark, 0.359 versus 0.555; and root xylem, 0.148 versus 0.324 per cent of sodium. Earlier studies on these plots by Aldrich *et al.* (1945) demonstrated that the inferior tree performance was caused primarily by poor water penetration (and moisture deficits) resulting from an accumulation of sodium in the exchange complex. The exchangeable calcium-to-sodium ratio in the calcium nitrate plots was 93:1, as compared with 8:1 in the sodium nitrate plots. Base exchange capacity of this soil varied between 5.23 and 8.35 meq/100 gm of soil. Exchangeable sodium in the sodium nitrate plots had built up to about 10 per cent of the exchange capacity. Salts had also accumulated at the 2-, 3-, and 4-foot levels in this soil as a result of lack of water penetration. Poor growth and yield of citrus in this orchard probably was not due as much to excessive sodium absorption by leaves and roots, as lack of adequate moisture for the trees.

Huberty and Pearson (1949) and Pearson and Huberty (1959), working with Washington navel orange trees irrigated for fifteen years with softened and natural Colorado River water and a local well water in which sodium was respectively 78, 40, and 28 per cent, found that yields were reduced by 15 per cent when irrigated with the softened (calcium replaced by sodium) Colorado River water. Valencia orange yields were only slightly reduced. These differences are almost too small to be considered significant, yet there was a definite trend toward lower yields when the softened water was used. The soil was a Ramona loam to clay loam, and the average annual rainfall was 14 inches. Soluble salts, particularly sodium and chloride, increased in the various soil horizons of plots treated with the softened Colorado River water over those irrigated with natural Colorado River water or well water. Exchangeable sodium as per cent total bases increased from values of 2.0 to 2.1 in plots irrigated with well water to values of 6.2 to 9.4 in plots irrigated with softened water. Sodium and chloride increased somewhat in leaves, feeder roots, root bark, and xylem of both orange varieties, but the values hardly seem high enough to account for decreased yields. It rather appears in this experiment that the increased total salt in the soil may have been a primary influence in decreasing navel orange yields. The navel orange leaves showed 0.16 versus 0.054 per cent of sodium in the dry matter. Saturation extract analyses made in the spring of 1949 (salt would be at minimal values in the spring after winter rains) showed average conductivity values of 1.9 and 3.04 mmho/cm for the upper 4 feet of soil.

Martin, Harding, and Murphy (1953) grew sweet and sour orange seedlings in potted soils of various exchangeable sodium percentages. The experimental soils were prepared as follows. Large batches of calcium-, magnesium-, potassium-, sodium-, and hydrogen-saturated soils were first prepared. Then samples of these were mixed to attain the proportions of exchangeable bases desired. The sodium series was prepared to contain sodium varying from < 1.0 to 28 per cent exchangeable sodium percentage (ESP). Yields of tops in grams were 60, 45, 36, 22, and 7 in a second crop of sour orange seedlings on a Yolo loam, which had < 1, 4, 7, 14, and 28 per cent ESP, respectively. The sodium content of the leaves of these seedlings were respectively 0.05, 0.21, 0.26, 0.40, and 0.78 per cent in the dry matter.

In further, similar experiments with Eureka lemon on several rootstocks, Jones *et al.* (1957) found on all rootstocks marked growth reductions at 13 per cent and drastic reductions at 28 per cent ESP. There were differences in the degree of growth reduction on different rootstocks. Greatest sodium uptake by leaves and leaf burn occurred in plants budded to Sampson tangelo and grapefruit. Best growth and lowest sodium uptake was with Cleopatra mandarin root. Best growth on all rootstocks and lowest sodium percentages in leaves were on the soils with an ESP of 1 or less. Increasing sodium decreased calcium and magnesium contents of leaves and increased potassium somewhat. All parts of the plant (leaves, stems, tap roots, and fibrous roots) reflected the varying sodium levels, but in general the spread between low and high sodium was greatest in the leaves.

In similar studies with trifoliate orange seedlings on soils of varying exchangeable sodium (and potassium) percentages, Martin *et al.* (1959) got growth reduction at 7 per cent ESP in a clay loam soil of exchange capacity of 25 meq/100 gm, and marked reduction at 15 per cent ESP. In a sandy loam of lower exchange capacity (10 meq/100 gm), growth reduction was much less at 7 and 15 ESP. Increases in sodium decreased calcium and increased potassium percentages in the leaves. These experiments indicate that trifoliate citrus seedlings may be more sensitive to sodium than some other citrus species.

In still further experiments of a similar nature with soils compounded to contain varying exchangeable sodium percentages under three soil conditions—slightly acid, base-saturated, and containing 1 per cent of calcium carbonate—Martin, Ervin, and Shepherd (1961) got a substantial yield reduction on one soil, at 6 per cent ESP, under all three of the aforementioned conditions. Where 0.1 per cent of VAMA was added to the soils, yield reductions were not as great. In these trials, feeder roots showed somewhat greater sodium differences in response to the lower ranges of ESP levels than the leaves. In general, the magnitude of growth reduction from increasing exchangeable sodium percentages was greater in base-saturated and limed soil than in slightly acid soil.

Haas (1952*a*, 1952*b*) grew Lisbon lemons on Brazilian sour root in outdoor soil cultures supplied with nutrient solutions varying in sodium content from 0 to 348 ppm and in calcium from 318 to 0. The highest sodium solution had the lowest calcium and vice versa. These solutions were made from sodium and calcium nitrate. Potassium sulfate and phosphate were also added to the nutrient solution. Growth was generally less at increasing sodium and decreasing calcium levels. Sodium in the leaves of plants depressed in growth showed values up to 0.17 per cent of sodium in the dry matter, and in these same high-sodium cultures rootlets showed between 0.56 and 0.75 per cent of sodium in the dry matter.

In further experiments, Haas and Brusca (1954*b*) added increasing amounts of sodium nitrate (in a modified Hoagland nutrient solution) to soil cultures of various citrus seedlings (Troyer citrange, grapefruit, Pomeroy trifoliate orange, Cleopatra mandarin, Spanish sour orange, rough lemon, tangelo, and sweet orange). The nutrient solution (of variable sodium nitrate content) was added periodically, and, in between, distilled water was added. No sodium injury symptoms or growth depression was noted. Leaf analyses for sodium, going from low to high sodium, showed values in the different seedlings from < 0.10 to 0.35 per cent in the dry matter. Highest sodium values in high-sodium cultures were found in Sampson tangelo seedlings and lowest in the sour orange. Root values for sodium showed a greater range in sodium going from low to high than did leaves. Lack of sodium injury in the cultures was probably due to the presence of ample calcium and magnesium and the diluting effect of distilled water additions to the soils.

Zusman (1956) grew sour orange and sweet lime seedlings in fertilized pots of soil to which sodium carbonate and sulfuric acid were added to produce pH values ranging from 4.40 to 9.02 in the soils growing sweet lime, and from 4.16 to 8.88 where sour orange seedlings were grown. All soils contained about 260 ppm of sodium. After some fifty days of growth, the sweet lime seedlings showed leaf burn, which from the description corresponded to sodium injury. Leaf analysis values ranged from 0.20 to 0.28 per cent of sodium in the dry matter. On the other hand, the sour orange seedlings growing under comparable conditions showed no leaf necrosis, and sodium levels in the leaves varied from 0.07 to 0.13 per cent in the dry matter.

Pearson (1951), working over a period of years in the author's laboratory and greenhouse, made an extensive investigation of sodium in relation to the behavior of lemon cuttings in carefully controlled sand and sand-bentonite mixtures. He determined effects on growth, appearance, and composition of (1) variable sodium concentrations under differing potassium, calcium, and magnesium levels, (2) variable anion carriers of sodium (chloride, sulfate, nitrate, and bicarbonate) with sodium maintained at 30 meq/l, and (3) sand-bentonite mixtures in equilibrium with nutrient solutions of variable sodium concentrations. In addition, he studied the usefulness of various plant parts as an indicator of sodium status.

Since these data have never been published, it appears worthwhile to record some of the principal results here.

All experiments were conducted in 3-gallon crocks of sand, flushed at frequent intervals with well-controlled nutrient solutions from large reservoirs. Iron was supplied by incorporating finely ground magnetite in the sand.

In the first experiment, increasing amounts of sodium chloride and sodium sulfate were added to a base nutrient solution to give sodium levels ranging from 0 to 20 meq/l. After four months of growth, no growth depression from the 20-meq/l sodium treatment was noted, and three higher sodium levels were added to the experiment, utilizing the cultures which during the initial period had received only 1, 3, and 5 meq/l sodium. At this time (December 16, 1944) two thirds of the plants were pruned back to the original cutting leaves and then grown for another year (with one additional severe pruning in April, 1945). The severe pruning after four months and again after one year caused plants which had hitherto not been affected by sodium at the 7-, 10-, 15-, and 20-meq/l levels to decrease in growth rate. The older leaves in the 20-meq/l treatment showed typical sodium burn injury and abscised. The weights of leaves from plants pruned off after the first four months and of the tops in the subsequent year of growth are shown in table 3–41, together with leaf analysis data.

The reduced growth and elevated sodium in leaves of pruned plants is no doubt a result of sodium being taken up by, or moved out of, roots into a much-reduced top, resulting in growth retardation and sodium buildup in the leaves. These results indicate that trees which are growing well or reasonably well in salt-affected soils may be badly injured by severe pruning, especially if adverse weather conditions (heat and wind) should coincide or shortly follow. Green, 4½-month-old leaves with no sodium burn, showed increasing sodium levels as growth decreased.

In another experiment under similar conditions, comparisons were made between equivalent amounts (30 meq/l) of sodium salts (sodium nitrate, sodium chloride, sodium sulfate, and sodium bicarbonate). The nature of the solutions used, growth, appearance, yield, and composition of leaves are shown in table 3–42. Growth was severely depressed by sodium bicarbonate, the plants developed iron chlorosis, and the leaves showed much sodium burn. The calcium content of leaves was greatly reduced. While all of the other salts depressed growth and there was burning and some shedding of older leaves, the plants were only moderately affected. Excluding the sodium bicarbonate culture, least growth depression occurred with sodium nitrate and greatest with sodium chloride. In all cases, the necrosis on older leaves was typical of sodium injury, and leaf values on 5½-month-old leaves were in the expected range of necrosis and growth depression. As in the first experiment, there was decreased calcium and magnesium in leaves of the growth-depressed plants, and there was increased potassium absorption.

In still another experiment carried out over thirteen months under comparable conditions, the effects of high levels of sodium at different calcium, magnesium, and total salt levels were

Table 3–41

GROWTH AND COMPOSITION OF LEMON CUTTINGS IN SAND CULTURES SUPPLIED WITH INCREASING AMOUNTS OF SODIUM

Sodium in Nutrient Solution (Meq/L)	Some Other Elements in Nutrient Solution (Meq/L)				Osmotic Pressure of Nutrient Solution (Atm)	Approx. EC of Nutrient Solution (Mmho/Cm)	First Harvest (Leaves Only); Planted June 6, 1944; Harvested Dec. 16, 1944					Growth and Composition (Plants Pruned Back Dec. 16, 1944 and April 16, 1945; Final Harvest Oct., 1945)							
	Ca	Mg	K	Cl			Growth and Leaf Appearance	Dry Weight of Leaves (Gm/Plant)	Leaf Composition (Per Cent in Dry Matter)			Growth and Leaf Appearance	Total Weight of Tops from Oct., 1944 to Oct., 1945 (Gm/Plant)	Leaf Composition of 4½-Month-Old Leaves (Per Cent in Dry Matter)					
									Ca	Na	Cl			Ca	Mg	K	N	Na	Cl
0	14	7	5	0	0.7	3.12	Excellent, green	11.0	2.68	0.03	0.04	Healthy, green	100.1	2.82	0.26	3.40	2.70	0.005	0.04
7	14	7	5	7	1.1	3.76	Excellent, green	10.9	2.82	0.04	0.25	Growth reduced, green	54.1	......	......	......	......	....	......
20	14	7	5	17.5	1.6	4.92	Excellent, green	10.5	2.55	0.06	0.32	Growth reduced, Na burn on a few leaves	52.8	2.27	0.21	4.93	2.27	0.27	0.36
30	14	7	5	22.5	1.8	5.72	....	......	......	......	......	Fairly healthy, but Na burn on some leaves	62.9	2.23	0.16	3.31	3.02	0.39	0.70
40	14	7	5	27.5	2.2	6.45	....	......	......	......	......	Reduced growth, considerable Na burn on leaves	34.9	2.5†	0.05	3.07	2.88	0.46	0.54
50	14	7	5	35	2.4	7.40	....	......	......	......	......	Reduced growth, severe Na burn	37.1	2.2†	0.17	3.50	......	0.99	0.96

Source: Pearson (1951).

† Analyses are of leaves showing typical sodium (Na) burn.

Table 3–42

GROWTH AND COMPOSITION OF LEMON CUTTINGS IN SAND CULTURES FROM JANUARY 24, 1950 TO AUGUST 15, 1950 AS AFFECTED BY VARIOUS SODIUM SALTS

Sodium in Nutrient Solution (Meq/L)	Other Principal Elements in Nutrient Solution (Meq/L)								EC of Nutrient Solution (Approx.) (Mmho/Cm)	Leaf Condition at End of Experiment	Yield, Average Dry Weight in 7 Months (Gm/Plant)	Composition of 5½-Month-Old Leaves (Per Cent in Dry Matter)			
	Ca	Mg	K	Cl	NO_3	SO_4	PO_4	HCO_3				Ca	Mg	K	Na
0	24.2	12.0	0.5	0	6.5	30.0	0.5	0	3.9	Healthy, green	128.1	4.32	0.38	1.30	0.009
30 (SO_4)	4.5	2.0	0.5	0	6.5	30.0	0.5	0	4.2	Green; some Na burning and bronzing	93.8	3.06	0.17	1.49	0.82
30 (Cl)	4.5	2.0	0.5	30.0	3.5	3.0	0.5	0	3.6	Green; some Na burning, bronzing, and leaf drop	73.0	2.95	0.18	1.64	0.75
30 (NO_3)	3.5	1.5	0.5	0	30.0	5.0	0.5	0	4.4	Green; some Na burning, bronzing, and leaf drop	100.6	3.12	0.21	1.56	0.96
30 (HCO_3)	4.5	2.0	0.5	0	3.5	3.0	0.5	30.0	3.8	Severely stunted, iron chlorosis, and Na burning	9.7	1.33	0.25	2.81	0.88

Source: Pearson (1951).

Table 3–43

GROWTH, APPEARANCE, AND LEAF COMPOSITION OF LEMON CUTTINGS GROWN IN SOLUTIONS OF VARIOUS SODIUM-DIVALENT ION RATIOS

Sodium as of Total Base in Nutrient Solution (Per Cent)	Tile No.	Composition of Nutrient Solution (Meq/L)								Approx. EC of Nutrient Solution (Mmho/Cm)	Growth and Leaf Appearance of Plants After 13 Months' Growth	Dry Weight of Tops After 13 Months Growth (Gm/Plant)	Composition of 4-Month-Old Leaves (Per Cent in Dry Matter)						
		Ca	Mg	K	Na	PO_4	NO_3	SO_4	Cl				Ca	Mg	K	Na	N	P	S
0	1	17.0	8.5	0.3	0	3.0	10.3	15.0	0	3.00	Plants vigorous and green	116.1	4.04	0.37	0.58	0.01	2.66	0.17	0.28
54	2	17.0	8.5	0.3	30.0	3.0	10.3	30.0	15.0	6.00	Slightly depressed growth, some Na burn on 1-year-old leaves	94.4	4.85	0.31	1.28	0.14	2.76	0.21	0.35
0	3	17.0	8.5	0.5	0	3.0	10.0	15.0	0	2.96	Plants vigorous and green	117.7	......	......	......	......	......	......	......
54	4	17.0	8.5	0.5	30.0	3.0	10.0	30.0	15.0	6.00	Slightly depressed growth, some Na burn on old leaves	85.9	......	......	......	......	......	......	......
52	8	1.0	0.5	0.5	2.2	0.5	3.0	1.0	0	0.54	Plants healthy, some sunburned	83.7	2.32	0.28	1.93	0.20	2.54	0.15	0.24
88	9	1.0	0.5	0.5	15.0	0.5	3.0	13.8	0	1.95	Depressed growth, some Na burn	64.4	1.04	0.08	4.83	0.61	2.68	0.17	0.34
94	10	1.0	0.5	0.5	30.0	0.5	3.0	28.8	0	3.62	Depressed growth, much Na burn, and yellowed leaves	35.0	1.18	0.21	2.32	0.94	2.87	0.19	0.33

Source: Pearson (1951).

studied. Data from this experiment are shown in table 3–43. Where calcium and magnesium were high, 30 meq/l sodium only slightly depressed total growth, and only a few older leaves showed sodium burn. Sodium in four-month-old leaves increased only slightly. The percentages of sodium as of total bases in the nutrient solution were 54 per cent (see data for tiles 2 and 4 in table 3–43).

In tiles 8, 9, and 10 (table 3–43), sodium varied from 2.2 to 30 meq/l and as per cent of total bases, from 52 to 94. Under the low calcium and magnesium conditions of these cultures, 15 and 30 meq/l of sodium markedly depressed growth, and there were corresponding increases in the sodium content of leaves and decreases in calcium and magnesium. Potassium, nitrogen, and sulfur also increased. Even though the exact physiological explanation of decreased growth is not possible, it is clear that under low calcium and magnesium conditions, increased sodium is much more disastrous in its effect than if the divalent cations are high.

In still further experiments, bentonite of high exchange capacity (113 meq/100 gm) was introduced into the 3-gallon sand cultures, and lemon cuttings were grown in plain sand and sand-bentonite mixtures at low- and high-sodium levels. It was found that the decreased permeability (and poor aeration) of the cultures to which 3 per cent bentonite was added (where the sodium saturation of the bentonite was 21 per cent) was more detrimental than the extra exchangeable sodium due to the presence of clay. Neither leaves nor feeder roots showed appreciably more sodium in the high-sodium, sand-bentonite mixture than in the high-sodium, sand-alone medium. These results are in harmony with those of other investigators working with other plants, namely, that the structural deterioration, pH increase, and related effects incidental to increasing sodium saturation are more adverse to plant growth than the direct effect on plant growth of the absorbed sodium per se.

The many chemical analyses made and the nature of the experimental setup in these experiments afforded an excellent opportunity to secure information on the plant part best suited to indicate sodium status. Analyses of leaves, stem, bark, stem wood, root back, root wood, and feeder roots of plants grown in control versus those receiving 30 meq/l sodium sulfate are shown in table 3–44. The data clearly indicate that mature leaves and feeder roots show the widest range in sodium content.

Table 3–44

SODIUM AND OTHER ELEMENTS IN VARIOUS PLANT PARTS
OF LEMON CUTTINGS GROWN IN NUTRIENT SOLUTIONS
OF 0 AND 30 MEQ/LITER SODIUM SULFATE

Sodium Status of Nutrient Solution	Plant Part	Leaf Composition (Per Cent in Dry Matter)				Ratio: Na in Treated Plants : Na in Control Plants
		Ca	Mg	K	Na	
None	Leaves (3 to 4 months old)	3.10	0.35	1.65	0.005	
30 meq/l Na_2SO_4	Leaves (3 to 4 months old)	1.77	0.15	2.31	0.70	140:1
None	Leaves (5 to 6 months old)	4.32	0.38	1.30	0.009	
30 meq/l Na_2SO_4	Leaves (5 to 6 months old)	3.06	0.17	1.49	0.82	89:1
None	Stem bark (5 to 6 months old)	3.45	0.33	0.77	0.12	
30 meq/l Na_2SO_4	Stem bark (5 to 6 months old)	3.98	0.40	0.84	0.26	2:1
None	Stem wood (5 to 6 months old)	0.15	<0.01	0.22	0.012	
30 meq/l Na_2SO_4	Stem wood (5 to 6 months old)	0.18	<0.01	0.18	0.18	15:1
None	Main root bark	2.07	0.42	0.71	0.013	
30 meq/l Na_2SO_4	Main root bark	2.22	0.30	1.11	0.17	13:1
None	Main root wood	0.10	Tr.	0.36	0.008	
30 meq/l Na_2SO_4	Main root wood	0.10	Tr.	0.36	0.11	13:1
None	Feeder roots	1.47	0.72	0.84	0.033	
30 meq/l Na_2SO_4	Feeder roots	0.59	0.45	1.34	1.96	59:1

Table 3–45

RELATION OF GROWTH TO LEAF SODIUM CONTENT AND INJURY OF THREE- TO SIX-MONTH-OLD LEMON TREES

Condition of Plant	Sodium in Leaves (Per Cent in Dry Matter)
Normal growth; no leaf symptoms	0.005– 0.15
Growth slightly reduced; sodium burn may appear on leaves older than 6 months (analyses are of nonburned leaves).	0.15 – 0.25
Growth moderately retarded; sodium burn on older leaves is prevalent (analyses are of nonburned leaves).	0.25 – 0.40
Growth considerably retarded; sodium burn may be severe on old leaves and some burn on younger leaves (burned leaves may show from 0.5 to >1.00 per cent sodium).	0.40 –>1.00

A summary showing the range of sodium in three- to six-month-old lemon leaves, as related to growth and presence or absence of sodium burn, is given in table 3–45. From these and data of other experiments reported, it appears that sodium values of less than 0.10 per cent in the dry matter can be considered as normal in mature leaves. Values in excess of this may indicate sodium troubles. Leaves with typical sodium burn will generally show values in excess of 0.70 per cent.

Estimation of the Exchangeable Sodium Percentage of Soils.—Since both exchangeable sodium and sodium in the soil solution influence plant growth and, in fact, are in equilibrium and because of difficulties in the exact determination of exchangeable sodium and ESP in some soils (particularly saline soils), Bower and Hatcher (1962) have suggested that the sodium absorption ratio (SAR) of an equilibrium extract may be more meaningful as a measure of the sodium status of a soil in its relation to plants than exchange sodium percentage (ESP). Sodium absorption ratio in soil extracts and irrigation waters is given by the following equation:

$$SAR = \frac{Na}{\sqrt{(Ca + Mg)/2}}$$

where the concentrations are expressed in milliequivalents per liter. However, more data are needed relating these values to citrus performance than are currently available.

Effect of Rootstock on Salt Absorption and Tolerance.—There is much evidence that the effects of salt on citrus are significantly affected by rootstock, variety, and other genetic factors. Cooper and his associates, working in Texas, have produced considerable information on this subject, and because of its practical importance a fairly complete summary of his investigations is recorded here.

Cooper (1948) grew Redblush grapefruit on sour orange and Cleopatra mandarin rootstocks in 15- by 15-foot basins irrigated with three waters. The control treatment was Rio Grande water, which ranged in electrical conductivity from 0.6

Table 3–46

GROWTH AND LEAF COMPOSITION OF RED BLUSH GRAPEFRUIT GROWN ON SOUR ORANGE AND CLEOPATRA MANDARIN WHEN IRRIGATED WITH WATERS OF VARYING SALT CONTENT

Salt in Irrigation Water (Ppm)	Rootstock	Growth Increase in Trunk Circumference (Cm)	Leaf Analyses (Per Cent in Dry Matter)			Leaf Condition
			Na	Cl	B	
Control	Sour orange	46.0	0.027	0.163	0.0076	No symptoms
Control	Cleopatra mandarin	40.2	0.039	0.085	0.0123	No symptoms
2,500	Sour orange	40.8	0.039	0.98	0.0086	Limited leaf bronzing on five trees
2,500	Cleopatra mandarin	39.6	0.041	0.24	0.0099	Limited leaf bronzing on two trees, but disappeared later
5,000	Sour orange	33.0	0.080	2.63	0.0093	Bronzing and necrosis of leaves and defoliation
5,000	Cleopatra mandarin	34.2	0.090	0.88	0.0119	Some bronzing of leaves

Source: Cooper (1948).

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to 1.4 mmho/cm or from approximately 380 to 840 ppm in total dissolved solids. The other two waters were treated with equal amounts of calcium chloride and sodium chloride to raise the salt content to about 2,500 to 5,000 ppm, respectively. Conductivity measurements made over the experimental period showed the waters varied from 3.0 to 4.8 mmho/cm for the medium salt content water and from 7 to 9 mmho/cm for the high salt content water. Repeated rains caused fluctuation in the salinity of the soils, but conductivity determinations on repeated saturation extracts made of samples of the first foot of soil gave values for the three plots as follows: river water, 0.8 to 2.0 mmho/cm; medium salt, 0.7 to 4.3; high salt, 0.6 to 7.0. Soil pH in all plots made on a paste ranged from 7.2 to 7.9. There was no pH difference between the various salt plots. The soil was a fine sandy loam.

It soon became evident that the grapefruit was much more severely affected by salt when on sour root than on Cleopatra mandarin root. Growth, as measured by increase in trunk circumference, and analyses of nine-month-old leaves are recorded in table 3–46. These data clearly show a greater chloride intake by the sour orange root and the appearance of more salt injury symptoms on the foliage.

In the same report, Cooper (1948) noted that Valencia orange on Cleopatra mandarin root was more tolerant to salt than Redblush grapefruit. He further noted that Cleopatra mandarin seedlings were more sensitive to chlorosis in highly calcareous soils than sour orange seedlings.

In a somewhat similar experiment with one-year-old Shary Red grapefruit on twenty different rootstocks, grown for 17 weeks in basins irrigated with water containing enough of a 50–50 mixture of calcium chloride and sodium chloride to produce a water of 4,000 ppm total salt, Cooper, Gorton, and Edwards (1951) reported the chloride content of a composite of spring-flush leaves picked on July 1 and September 22, 1950, and also noted the extent of injury. The data reported are shown in table 3–47.

It is evident that least injury occurred on *Severinia buxifolia*, Cleopatra mandarin, and Rangpur lime rootstocks, and greatest injury on stocks of trifoliate orange, citron, and Florida sweet orange. Sour orange was intermediate, as were several of the tangelos and sweet oranges.

Further data from similar experiments reported by Cooper (1962) are shown in table 3–48.

Table 3–47

SEVERITY OF SALT EXCESS INJURY AND CONCENTRATION OF CHLORIDES IN LEAVES OF SHARY RED GRAPEFRUIT ON DIFFERENT ROOTSTOCKS IN PLOTS IRRIGATED WITH A SOLUTION CONTAINING 4,000 PPM SALT

Rootstock Variety	Severity of Injury	Per Cent Chloride in Leaves, Dry-Weight Basis
Plot A: concentration of salts in saturation extract of soil: 2,800 ppm*		
Sour orange	Slight	1.8
Williams tangelo	Slight	1.4
Minneola tangelo	Slight +	1.5
Sampson tangelo	Moderate	1.6
Florida sweet orange	Severe	2.5
Plot B: concentration of salts in saturation extract of soil: 2,500 ppm*		
Sour orange	Moderate +	2.5
Pineapple orange	Moderate +	2.4
Cuban shaddock	Moderate	2.3
Nakorn pummelo	Moderate +	1.8
Duncan grapefruit	Slight +	1.7
Plot C: concentration of salts in saturation extract of soil: 3,010 ppm*		
Sour orange	Slight +	2.4
Rough lemon	Slight	1.4
Rangpur lime	Very slight	0.4
Sweet lemon	Slight +	1.9
Columbian sweet lime	Slight +	2.6
Plot D: concentration of salts in saturation extract of soil: 2,380 ppm*		
Sour orange	Slight +	1.8
Cleopatra mandarin	Very slight	0.7
Calamondin	Slight +	2.1
Citrus nobilis	Slight	1.6
Rusk citrange	Moderate	2.9
Plot E: concentration of salts in saturation extract of soil: 2,660 ppm*		
Sour orange	Slight	1.5
Trifoliate orange	Severe +	2.7
Severina buxifolia	No injury	0.6
Citron	Very severe	3.5
Citrumelo No. 4475	Slight	1.9

Source: Cooper *et al.* (1951).

* Determinations made on soil samples of the upper two feet of soil, taken on July 21, 1950.

The results confirm other data in showing the salt tolerance of Rangpur lime and Cleopatra mandarin compared with the salt susceptibility of rough lemon, tangelos, sour orange, pummelo, citrange, and trifoliate orange. Also good relations between leaf burn and chloride and sodium content of leaves are shown by these data.

Continuing experiments with Shary Red

Table 3–48

CHLORIDE AND SODIUM IN LEAVES AND MANIFESTATIONS OF TOXICITY IN RUBY RED GRAPEFRUIT TREES ON VARIOUS ROOTSTOCKS GROWN IN CONTROL AND SALINIZED PLOTS

	Control Plots†			Salinized Plots‡		
Kind of Rootstock†	Toxicity Symptoms*	Cl in Dried Leaves (Per Cent)	Na in Dried Leaves (Per Cent)	Toxicity Symptoms*	Cl in Dried Leaves (Per Cent)	Na in Dried Leaves (Per Cent)
Rangpur lime	None	0.10	0.11	None	0.42	0.09
Severinia buxifolia	None	0.25	0.09	None	0.64	0.09
Cleopatra mandarin	None	0.11	0.05	None	0.64	0.07
Rough lemon	None	0.18	0.07	Leaf burn	1.45	0.09
Minneola tangelo	None	0.21	0.16	Leaf burn	1.53	0.25
Sampson tangelo	None	0.14	0.14	Leaf burn	1.63	0.23
Sour orange	None	0.28	0.05	Leaf burn	1.81	0.09
Nakorn pummelo	None	0.25	0.05	Leaf burn	1.81	0.05
Calamondin	None	0.39	0.14	Defoliation	2.16	0.32
Cuban shaddock	None	0.46	0.05	Defoliation	2.34	0.16
Florida sweet orange	None	0.75	0.05	Defoliation	2.52	0.11
Rusk citrange	None	0.75	0.05	Twig necrosis	2.90	0.09
Etrog citron	Leaf burn	0.89	0.18	Twig necrosis	3.50	0.31
Trifoliate orange	Leaf burn	0.90	0.18	Twig necrosis	§	......

Source: Cooper (1962).

* After 7 weeks of treatment.

† Irrigated with Rio Grande River water. The saturation extract of the soil contained 990 ppm total soluble salts.

‡ Irrigated with 4,000 ppm NaCl + $CaCl_2$ salt solution. The saturated extract contained 3,300 ppm total soluble salts.

§ No leaves present on the tree.

grapefruit and Valencia oranges on sour orange and Cleopatra mandarin rootstocks were reported by Cooper, Gorton, and Olson (1952), and again salt injury and chloride uptake was much less with Cleopatra rootstock than with sour orange. This experiment also involved boron variables. This element (unlike chloride) was absorbed in significantly greater amounts by the trees on Cleopatra than on sour orange. The relative absorption of salt by leaves, twigs, bark, trunk wood, and roots in the high- and low-salt treatments was reported. The leaves were the best indicator of high chloride in the soil and also showed somewhat elevated sodium levels, but the taproot and lateral and fibrous roots were a little better indicators of sodium levels in the high-salt plots. Leaves were the best indicators of varying boron status.

In studies with young nucellar-line Redblush grapefruit trees, Cooper and Peynado (1959*b*) reported on the growth from April 24 to November 19, 1958, and on leaf composition and appearance with respect to chlorine and boron when trees were irrigated with a saline well water containing 3,300 ppm total solids and 5.5 ppm boron. Thirteen rootstocks were used. The sodium percentage of the water was 85. The planting was irrigated seven times during the test period. Data from the experiment are shown in table 3–49.

These data show that much less chloride was accumulated from the mandarin stocks and Rangpur lime than from the citrange, citrumelo No. 4475, *Citrus macrophylla,* and *Citrus moi* stocks. However, the order of accumulation for boron was different from that of chloride.

In this same paper, Cooper and Peynado (1959*b*) reported on the growth, chloride accumulation, and severity of chloride-toxicity symptoms of young-line and old-line Valencia orange trees on sour orange rootstock, irrigated with waters of increasing sodium chloride content. Each young tree was planted in rows 5 feet apart and irrigated in 36-inch-diameter circular plots, separated from surrounding soil by galvanized iron barriers inserted in the soil to a depth of 30 inches. The single-tree plots were irrigated four times with 4 acre-inch equivalents of the various waters from June 24 to September 3, 1958. Data on this experiment are reported in table 3–50 and show marked differences in chloride accumulation by young- and old-line scions.

In further research with Webb Redblush grapefruit trees on sour orange and Cleopatra mandarin rootstocks, Cooper *et al.* (1958) grew trees for four years in small basins differentially

Table 3–49

EFFECT OF ROOTSTOCK ON GROWTH AND TOXICITY SYMPTOMS OF CHLORIDES AND BORON IN YOUNG-LINE RED BLUSH GRAPEFRUIT TREES PLANTED FEBRUARY 18, 1958, AND IRRIGATED WITH 28 ACRE-INCHES OF WELL WATER BETWEEN MARCH AND SEPTEMBER, 1958*

Kind of Rootstock	Cross-Sectional Area of Trunk on April 24, 1958 (Cm^2)	Increase in Cross-Sectional Area of Trunk from April 24 to Dec. 19, 1958 (Cm^2)	Boron Content of Leaves on Aug 6, 1958 (Ppm)	Chloride Content of Leaves on Aug. 6, 1958 (Per Cent)	Severity of Toxicity Symptoms on Dec. 19, 1958 and Apparent Cause†	
Sour orange	174‡	105‡	786‡	0.46‡	3.3‡	Boron
Cleopatra mandarin	194	66	1,134	0.22	4.0	Boron
Timkat mandarin	950	26	899	0.12	4.5	Boron
Ponkan mandarin	406	20	1,174	0.66	4.5	Boron
Sunki mandarin	1,825	81	787	0.10	4.0	Boron
Carrizo citrange	140	33	742	2.11	4.0	Chloride
Savage citrange	652	31	939	1.34	4.5	Chloride
Troyer citrange	364	32	873	2.43	4.5	Chloride
Citrumelo No. 4475	121	158	904	0.83	4.5	Boron
Citrus macrophylla	35	92	282	0.67	0	....
Citrus moi	361	168	521	1.69	1.3	Chloride
Columbian sweet lime	2,987	123	762	0.79	3.3	Boron
Rangpur lime	2,819	95	854	0.40	4.3	Boron

Source: Cooper and Peynado (1959*b*).

* Twenty-four inches of rain fell during September, October, and November, 1958.

† Most of the trees showed combined boron- and chloride-toxicity symptoms. However, where chlorides were accumulated in excess, the leaves burned and dropped off by midsummer. Boron toxicity, on the other hand, was a slower process. The leaves first showed yellow dots, then orange mottling and tip burn, and finally defoliation in late fall and winter. Where chloride accumulation was only moderately high, bronzing was frequently the only symptom that developed; but when boron mottling was superimposed on bronzing, it was difficult to distinguish the two symptoms. The key used for scoring of severity of toxicity symptoms was as follows: 1 = some yellow dots and/or slight bronzing; 2 = numerous yellow dots, orange mottling of leaf tip, and/or moderate bronzing; 3 = prominent orange mottling and/or bronzing; 4 = leaf necrosis; and 5 = defoliation and twig necrosis.

‡ The values given represent the mean of four single-tree replicates.

Table 3–50

EFFECT OF SODIUM CHLORIDE (NaCl) ADDITIONS TO IRRIGATION WATER (16 ACRE-INCHES APPLIED BETWEEN JUNE AND SEPTEMBER, 1958) ON GROWTH, CHLORIDE ACCUMULATION, AND CHLORIDE TOXICITY SYMPTOMS ON YOUNG-LINE AND OLD-LINE VALENCIA ORANGE TREES PLANTED FEBRUARY 18, 1958*

NaCl Treatment and Valencia Orange Line	Cross-sectional Area of Trunk on June 24, 1958 (Cm^2)	Increase in Cross-Sectional Area of Trunk from June 24 to Dec. 19, 1958 (Cm^2)	Chloride Content of Leaves (Per Cent)		Severity of Chloride Toxicity Symptoms on Dec. 19, 1958†
			Aug. 15, 1958	Nov. 13, 1958	
Base river water control:					
Young line	162	258	0.07	0.12	0
Old-line	163	151	0.18	0.13	0
3,000 ppm NaCl:					
Young-line	174	213	1.10	1.06	0.8
Old-line	155	131	1.24	1.02	0.5
4,000 ppm NaCl:					
Young-line	200	184	1.06	0.86	0.8
Old-line	188	120	1.30	1.27	1.2
5,000 ppm NaCl:					
Young-line	176	182	1.68	1.44	3.0
Old-line	180	96	1.87	1.69	2.2

*Source:*Cooper and Peynado (1959*b*).

* Twenty-four inches of rain fell during September, October, and November, 1958.

† Key to scoring chloride toxicity: 0 = no symptoms; 1 = slight bronzing; 2 = moderate bronzing; and 3 = severe bronzing and slight necrosis.

irrigated with a low-salt-content river water and a highly saline, high-sodium well water. (The soil was a fine sandy loam, well drained, with no water-table problem.) The soil was further modified by additions of ammonium nitrate, calcium sulfate, and calcium nitrate. Data on this experiment are condensed in table 3–51.

The composition of the irrigation waters used and the effects of two years' application on the salt content of the first foot of soil show: (1) electrical conductivity values well above those thought safe for citrus, and (2) major increases in the sodium and chloride of the saturation extract.

Growth of trees on both rootstocks was depressed when the well water was used, but in most cases more so on sour orange than on Cleopatra stock. Also, visual symptoms of both boron and salt injury, in general, were worse where trees were on sour stock. The addition of calcium nitrate to the high-boron-content well water markedly reduced the amount of boron injury symptoms and to some extent the boron uptake, as measured by leaf analysis.

In spite of the rather high sodium in the first foot of soil (where most of the roots of these trees were growing), this element was not excessively absorbed. No doubt this was due in part to the presence of considerable calcium, since calcium exerts a powerful blocking effect on sodium uptake. As in previous experiments, chloride uptake was appreciably less on Cleopatra than on sour orange stock.

The growth depression in these trees is probably due to the influence of (1) high-salt content of the soil solution (osmotic effects), and (2) excessive chloride and boron absorption.

These data, aside from the rootstock effect, are of especial interest in showing that in spite of the use of a high-salt and boron-content water (approximately 3,300 ppm) reasonably good growth and not much salt or boron injury was evident during the four years of this experiment when Cleopatra mandarin root was used along with generous applications of calcium nitrate. The latter definitely lowered boron uptake and probably had a beneficial balancing affect on sodium, though this is not reflected in the leaf analyses reported. Annual rainfall during the four years of this experiment ranged from 14 to 22 inches. This undoubtedly reduced salt accumulation considerably. Total irrigation water applied was approximately 144 acre-inch equivalents for the four-year period. Rain plus irrigation for the four years was as follows: 1953, 49 inches; 1954, 54 inches; 1955,

Table 3–51

GROWTH AND CHEMICAL COMPOSITION OF YOUNG WEBB RED BLUSH GRAPEFRUIT ON SOUR ORANGE AND CLEOPATRA MANDARIN ROOTSTOCKS IN RELATION TO WATER QUALITY

	River Water		Well Water			
Type of Observation or Measurement	No Addition	Plus $CaSO_4$	No Addition	Plus NH_4NO_3 at 1.09 Gm/L	Plus $CaSO_4$ at 3.8 Gm/L	Plus $Ca(NO_3)_2$ at 3.6 Gm/L
Experiments started June 14, 1953:						
EC (mmho/cm)*	1.3	2.8	5.5	7.5	7.0	7.2
SAR	4.8	2.0	28.0	30.0	13.0	12.0
B (ppm)	0.5	0.5	6.9	6.8	6.9	6.7
Soil analysis, saturation extract (0 to 12 inches, Aug. 16, 1955; original soil pH 7.3; base exchange capacity 10.7 meq/100 gm):						
EC (mmho/cm)	1.3	3.7	6.0	6.5	5.9	6.5
SAR	3.7	0.6	27.6	30.9	13.7	11.8
B (ppm)	1.9	2.6	6.3	6.0	6.9	6.7
Na (meq/1)	6.6	2.3	52.7	53.9	41.0	41.0
Ca + Mg (meq/l)	6.4	34.7	7.3	6.1	18.0	24.0
Cl (meq/l)	6.1	6.1	33.8	33.9	31.9	33.9
SO_4 (meq/l)	5.0	36.0	16.6	17.0	37.3	17.6
N (meq/l)	0.4	0.4	....	27.4	....	30.7
Growth of trees, cross-section of trunk, as of Nov. 15, 1956 (cm^2):						
On sour orange root	23.25	24.15	19.10	19.53	19.03	22.25
On Cleopatra mandarin root	26.65	24.70	22.38	21.22	25.87	22.08
Visual leaf symptoms of boron excess† at end of experiment, Nov. 1, 1956:						
On sour orange root	0	0	1.8	3.0	1.0	0.5
On Cleopatra mandarin root	0	0	0.3	0.5	0.1	0.1
Boron in leaves on Aug. 17, 1956 (ppm in dry matter):						
On sour orange root	151.0	171.0	1,097.0	1,323.0	1,043.0	912.0
On Cleopatra mandarin root	168.0	170.0	1,211.0	1,566.0	942.0	871.0
Visual leaf symptoms of salt excess‡ at end of experiment, Nov. 11, 1956						
On sour orange root	0	0	1.8	3.0	1.0	0.5
On Cleopatra mandarin root	0	0	0.3	0.5	0.1	0.1
Leaf analysis values for spring-flush leaves picked on Aug. 17,1955 (per cent in dry matter):						
Na on sour orange root	021	0.21	0.33	0.32	0.25	0.31
Na on Cleopatra mandarin root	0.25	0.26	0.43	0.43	0.35	0.38
K on sour orange root	1.54	1.54	1.58	1.64	1.51	1.32
K on Cleopatra mandarin root	1.28	1.20	1.14	1.13	1.28	1.00
Ca on sour orange root	4.81	5.01	4.89	4.60	5.14	4.90
Ca on Cleopatra mandarin root	4.48	4.84	4.60	4.40	5.15	4.86
Mg on sour orange root	0.30	0.32	0.29	0.28	0.30	0.26
Mg on Cleopatra mandarin root	0.40	0.44	0.43	0.40	0.44	0.40
Cl on sour orange root	0.11	0.11	0.42	0.44	0.62	0.41
Cl on Cleopatra mandarin root	0.14	0.19	0.26	0.27	0.23	0.23
SO_4 on sour orange root	0.72	1.87	0.62	0.26	0.87	0.18
SO_4 on Cleopatra mandarin root	0.67	1.75	0.74	0.52	1.52	0.34
N on sour orange root	2.21	2.18	2.23	2.71	2.22	3.03
N on Cleopatra mandarin root	2.41	2.24	2.28	2.62	2.24	2.74

*Source:*Cooper *et al.* (1958).

* Mmho/cm × 640 gives an approximation of parts per million.

† Key: 0= no boron excess symptoms; 1 = scattered yellow spots; 2 = half of leaves on tree showed mottling; 3 = all leaves mottled and slight tip burn; and 4 = some defoliation of mottled leaves.

‡ Key: 0 = no salt excess symptoms; 1 = defoliation of apparently normal leaves; 2 = twig terminals showed some necrosis; 3 = considerable twig necrosis; and 4 = whole branches killed.

58 inches; and 1956, 54 inches. With the type soil and prevailing climate, considerable root-zone leaching no doubt occurred and thus reduced salt buildup in the root zone.

A paper published by Cooper *et al.* (1956) summarizes observations for a number of years on disease, salt, boron, lime, and cold tolerance of grapefruit on numerous rootstocks. These observations are reproduced in table 3–52. Although they refer to conditions in Texas, the data have much wider application. Very little other field data of the scope and character of Cooper's work have come to the author's attention.

In Australia, Smith (1963) compared the chloride content of Washington navel oranges grown on Citronelle (rough lemon) and trifoliate roots. Much more chlorine accumulated in the leaves of plants grown on trifoliate roots.

Salt Injury From Sprays.—Foliar absorption of salt from overhead or underhead irrigation and ocean sprays and mists has been noted. Citrus leaf injury from the use of underhead sprinkler irrigation systems, in which only the skirts of the trees were wetted, was noted in several areas of California by Harding, Miller, and Fireman (1958). They found an appreciable accumulation of sodium and chlorine in the sprinkled lower leaves of grapefruit, orange, and mandarin orchards where waters had been used with total salt content ranging from 491 to 931 ppm of total soluble solids. Data from this study are shown in table 3–53.

In further studies of foliar absorption by citrus, Eaton and Harding (1959) found that Valencia orange trees, sprinkled intermittently with a water containing 969 ppm total salt (163 sodium, 135 chlorine, and 384 sulfate) for six 9-hour periods, absorbed substantial amounts of sodium and chlorine. Absorption was much greater when sprinkling was done in the daytime than at night. In addition to increased sodium and chloride absorption, there was a significant increase in sulfur in the leaves. Absorption was much greater when the sprinkling was intermittent rather than continuous, the same total sprinkling time being used in both cases.

Ehlig and Bernstein (1959) also conducted sprinkling trials with avocado, navel oranges, almond, apricot, plum, alfalfa, beans, cabbage, clover, lettuce, onion, spinach, tall fescue, and tomato, using various salt solutions of different concentrations. All plants were grown in sand cultures. The vegetable and fields crops showed little injury, even with waters containing up to 96 meq/l of sodium chloride. Of the trees, all but avocados absorbed enough salt to be injurious to the leaves. Orange leaves lost their gloss, and some showed tip burn when sprinkled intermittently with waters containing from 5 to 10 meq/l of calcium chloride, sodium chloride, or sodium sulfate for 42 hours of total sprinkling time. Injury was worse from daytime than nighttime sprinkling. Partial to complete defoliation occurred with almond, plum, and orange sprinkled with high sodium chloride and calcium chloride solutions (10 meq/l) intermittently for 59 hours in the daytime. Injured orange leaves showed from 0.70 to 1.05 per cent of chlorine and 0.46 to 0.69 per cent of sodium.

Miwa, Gomi, and Yamamoto (1957) found that defoliation of citrus trees from briny sprays could be reduced by a spray of 2,4–D at concentrations of 50 to 100 ppm. Noro (1956) obtained similar results.

Mungomery (1959), in Australia, has noted injury to citrus from overhead sprinklers where the application rate was so slow that leaves (in very hot weather) dried off between each rotation of the sprinkler. Early morning, late evening, or night irrigation was recommended as a means of reducing salt injury.

Smith (1963), working in Western Australia, conducted spraying experiments with Washington navel oranges somewhat similar to those reported by Eaton and Harding (1959). Comparable results were obtained.

By way of general summary on salinity as related to citrus performance, it is evident from present information that no exact limit can be placed on permissible salt levels in the soil. For general guidance purposes, it would appear that when chloride in the root zone of any citrus soil exceeds 70 ppm (expressed on a dry soil basis) or exceeds 350 mgm/l (10 meq) in a saturation extract, the possibility exists for some chloride injury, though severe injury is not likely with values reaching several times these concentrations.

With respect to sodium status, (1) exchangeable sodium percentage (ESP); (2) concentration of sodium in the soil solution, and (3) accompanying cations (especially calcium) and anions (especially bicarbonate and chloride) make it difficult to set limits. However, the evidence suggests that when the ESP exceeds 6.0 in any part of the root zone, the possibility exists for decreased permeability, aeration, nutrient availabil-

Table 3–52

CLASSIFICATION AS TO DISEASE, SALT, BORON, LIME, AND COLD TOLERANCE OF CITRUS VARIETIES USED IN TEXAS AS ROOTSTOCK FOR WEBB RED BLUSH AND SHARY RED GRAPEFRUIT

Group and Variety Used as Rootstock	Tolerant to Tristeza in Brazil*	Symptoms of Indicated Disease Visible	Extent of Tolerance†			
			Salt	Boron	Lime	Cold
Mandarin:						
Cleopatra	Yes	None	gt	‡	mt	mt
Sunki	Yes	Bark shelling, cause unknown	gt	§	mt	mt
Timkat	......	....	gt	....	pt	....
Suenkat	Yes	Cachexia (many)	mt	....	pt	mt
Ponkan	Yes	None	mt	mt	pt	mt
Betangas	......	....	mt	....	pt	pt
Dancy	Yes	Cachexia (few)	mt	....	mt	mt
Kunembo	......	....	gt	....	mt	....
Oneco	Yes	Cachexia (few)	mt	....	pt	mt
Clementine	Yes	Cachexia (few)	mt	pt	pt	pt
Lau Chang	......	None	mt	pt	pt	mt
Changsha	......	None	mt	....	pt	mt
Pong Koa PI 14054	Yes	Cachexia (few)	mt	....	pt	mt
Chu Koa PI 10032	Yes	Cachexia (few)	mt	....	pt	mt
Sanguinea	......	Cachexia (few)	gt	....	....	pt
Choo Chou Tien Chieh	Yes	Cachexia (many)	pt	....	....	pt
Willowleaf	......	Cachexia	mt	....	mt	pt
Miray	......	....	pt	....	pt	pt
C. *nobilis* PI 10642	Yes	....	pt	....	pt	pt
Silverhill satsuma	......	Cachexia	pt	....	pt	pt
False hybrid satsuma	......	Cachexia	pt	....	pt	pt
Shekwasha ‖	......	....	mt	....	gt	mt
Calashu#	......	....	mt	gt	....	mt
Tangor:						
Umatilla	Yes	Cachexia (few)	....	....	pt	mt
Altoona	......	Cachexia (few)	pt	....	....	mt
Tangor CPB653	Yes	....	mt	....	....	pt
King	Yes	None	pt	....	mt	pt
Kara	Yes	None	pt	....	pt	pt
Kinnow	Yes	None	mt	....	pt	mt
Temple	Yes	Cachexia (few)	....	....	pt	mt
Tangelo:						
San Jacinto	Yes	None	mt	§	....	mt
Webber	Yes	None	mt	mt	....	mt
Williams	Yes	Bark shelling, cause unknown	mt	pt	mt	mt
Orlando	Yes	Cachexia (many)	mt	....	pt	pt
Sunshine	Yes	Cachexia (many)	mt	mt	pt	mt
Suwannee	Yes	Cachexia (many)	mt	....	pt	mt
Watt	No	None	mt	pt	pt	mt
Thornton	No	Cachexia (many)	mt	pt	pt	mt
Minneloa	Yes	Cachexia (many)	mt	pt	mt	mt
Pina	No	Cachexia (many)	mt	pt	pt	mt
Yalaha	Yes	Cachexia (many)	mt	....	pt	mt
Seminole	Yes	Cachexia (many)	mt	....	mt	pt
Sampson	Yes	None	mt	pt	pt	mt
Pearl	......	....	pt	....	....	pt
Sour lime:						
Mexican	No	....	mt	mt	mt	pt
Egyptian sour	......	....	mt	....	mt	pt
Kalpi	No	None	mt	....	pt	pt

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Table 3–52 (Continued)

Group and Variety Used as Rootstock	Tolerant to Tristeza in Brazil*	Symptoms of Indicated Disease Visible	Extent of Tolerance†			
			Salt	Boron	Lime	Cold
Sweet lime:						
Palestine	Yes	Xyloporosis	pt	pt	mt	mt
Columbian	Yes	Xyloporosis	mt	pt	mt	mt
Butnal	......	Xyloporosis	mt	pt	mt	mt
Mandarin lime:						
Rangpur	Yes	RLD**; cachexia (few)	gt	mt	gt	gt
Kusaie	......	RLD; cachexia (few)	gt	....	....	....
Poak Ling Ming	......	RLD; cachexia (few)	gt	....	....	....
Ling Ming	Yes	RLD; cachexia (few)	gt	....	....	....
Lemon:						
Rough lemon	Yes	None	mt	mt	gt	mt
Kumquat hybrid:						
Winter Haven lemonquat	......	Bud-union crease	pt	pt	....	mt
Tavares limequat	......	Bud-union crease	....	....	....	pt
Lakeland limequat	......	....	....	....	....	pt
Calamondin	......	Bud-union crease	mt	pt	mt	mt
Trifoliate orange:						
Trifoliate orange	Yes	Exocortis	pt	mt	pt	pt
Citrange:						
Rusk	Yes	None	pt	mt	pt	pt
Troyer	Yes	None	pt	gt	pt	pt
Savage	Yes	None	mt	mt	mt	mt
Carrizo	......	....	mt	§	mt	....
Morton	Yes	Exocortis	....	gt	mt	....
Cunningham	Yes	Exocortis	pt	....	pt	....
Saunders	......	None	pt	gt	pt	mt
Uvalde	No	None	pt	gt	pt	pt
Norton	......	....	pt	gt	pt	pt
Rustic	......	None	pt	gt	....	pt
Citrumelo:						
Sacaton	Yes	None	mt	mt	pt	mt
Citrumelo CPB4475	Yes	None	mt	mt	pt	mt
Other trofoliate orange hybrids:						
Highgrove citremon	No	None	mt	....	....	mt
Brownell citradia	......	Exocortis	pt	....	....	mt
Thomasville citrangequat	......	None	pt	....	pt	pt
Glen citrangedin	......	Bud-union crease	pt	....	....	mt
Citrangor CPB44301-A_2	......	None	pt	mt	....	pt
Citraldin CPB50130	......	None	mt	....	....	mt
Citranguma CPB48060	......	None	mt	....	....	mt
Sweet orange:						
Pineapple	Yes	Foot rot	mt	gt	pt	mt
Hamlin	Yes	Foot rot	mt	....	pt	mt
Florida	Yes	....	pt	....	pt	mt
Louisiana	......	Foot rot	mt	....	pt	mt
Torregrossa	Yes	Foot rot	mt	....	pt	mt
Gzel Gzel	......	Foot rot	mt	....	pt	mt
Lamb summer	Yes	Foot rot	mt	....	pt	mt
Weldon	......	Foot rot	mt	....	pt	mt
Valencia	Yes	Foot rot	mt	....	pt	mt
Precoce	......	None	mt	....	pt	mt
Maltese Oval	......	....	....	....	pt	mt
Avena Blood	......	Foot rot	mt	....	pt	mt

Table 3–52 (Continued)

Group and Variety Used as Rootstock	Tolerant to Tristeza in Brazil[*]	Symptoms of Indicated Disease Visible	Extent of Tolerance[†]			
			Salt	Boron	Lime	Cold
Caderna	......	Foot rot	mt	....	pt	mt
Indio	......	Foot rot	mt	....	....	mt
Sanguinea Grosse Ronde	§	....	....	....	pt	....
Harvard No. 2	......	....	....	....	pt	....
Drake Star	......	....	....	....	pt	....
Enterprise	Yes	....	....	....	pt	....
Homosassa	Yes	....	mt	gt	pt	mt
Sour orange:						
Texas	No	None	mt	mt	gt	mt
Florida	No	None	mt	mt	gt	mt
Bergamot	No	None	mt	....	gt	mt
Sauvage	No	None	mt	....	gt	mt
Bittersweet	No	None	mt	....	gt	mt
Oklawaha	No	None	mt	....	gt	mt
Grapefruit:						
Duncan	No	None	mt	mt	pt	mt
Webb Redblush	No	None	....	mt	pt	mt
Leonardy	No	Cachexia (many)	....	....	mt	mt
Shaddock:						
Siamese	No	None	mt	....	pt	mt
Thong Dee	No	None	mt	....	pt	mt
Cuban	No	None	mt	pt	mt	mt
African	......	None	mt	pt	....	mt
Other species:						
Severinia buxifolia	No	None	gt	gt	pt	gt
Citron PI 11292	......	Weak root system	pt	pt	....	pt
Yuzu ichandarin[††]	......	Bud-union crease	....	....	....	....
Natsu Mikan[‡‡]	No	None	pt	....	pt	pt

Source: Cooper *et al.* (1956).

[*] Based on work in Brazil (Grant and Costa, 1949; Grant, Costa, and Moreira, 1950). No tristeza was found in any of the experimental orchards in Texas.

[†] Key: gt = good tolerance; mt = moderate tolerance; pt = poor tolerance.

[‡] Boron tolerance of Cleopatra between moderate and poor tolerance.

[§] Indicates no determination.

[||] Shekwasha is considered to be a natural hybrid (***Citrus tachibana*** × mandarin).

[#] Calashu is a hybrid of Calamondin and satsuma.

[**] Rangpur lime disease.

[††] Considered to be a natural hybrid (*Citrus ichangensis* × sour mandarin).

[‡‡] A sour orange type.

ity, and the chain of other consequences which follow.

In the soil solution or saturation extract, soluble sodium in excess of 20 meq/l (particularly if calcium and magnesium make up less than 50 per cent of the soluble cations) may cause some growth depression, especially if the prevailing anion is chloride. Serious trouble may be expected if sodium bicarbonate is found in amounts much under 20 meq/l.

With respect to total soluble salt, the best estimate at present is to be had from a measure of the electrical conductivity of a saturation extract. Values in excess of EC 2.0 mmho/cm should be regarded with suspicion. As shown by Cooper's data, however, the temporary presence of higher salt levels may not be injurious, and rootstocks such as Cleopatra mandarin, Rangpur lime, and others may achieve reasonably good citrus performance under much higher salt conditions than the value now regarded as permissible.

With respect to irrigation water quality, barring severe climatic extremes, such as sudden temperature peaks, desiccating winds, and frosts, and with the use of tolerant rootstock, adequate root-zone leaching, good care and fertilization,

Table 3–53

CHEMICAL ANALYSES OF LEAVES FROM UPPER (NONWETTED) AND LOWER (WETTED) PARTS OF CITRUS TREES IN LOW-HEAD SPRINKLED ORCHARDS IN RELATION TO COMPOSITION OF IRRIGATION WATER USED

California Location	Source	Irrigation Water Composition (Ppm)									Kind of Citrus or Variety	Position on Tree	Leaf Composition (Per Cent in Dry Matter)						
		B	NO_3	HCO_3	SO_4	Ca	Mg	Cl	Na	Total Salts			Ca	Mg	K	Na	Cl	S	B
Hemet	Well	0.10	19	195	264	98	22	75	95	768	Grapefruit	Upper	4.7	0.43	0.53	0.13	0.12	0.45	0.010
												Lower	4.7	0.50	0.37	0.63	0.59	0.44	0.011
La Sierra	Well	0.10	86	305	134	78	7	131	190	931	Valencia orange	Upper	5.6	0.36	0.48	0.17	0.04	0.33	0.007
												Lower	5.6	0.40	0.41	0.53	0.16	0.30	0.0075
Corona	Well	0.20	17	231	154	114	13	117	69	715	Navel orange	Upper	5.4	0.28	0.45	0.04	0.11	0.35	0.0080
												Lower	5.3	0.25	0.30	0.41	0.36	0.32	0.0090
Coachella	Colorado River	0.16	Tr.	146	375	88	34	124	145	912	Tangerine	Upper	4.0	0.22	1.07	0.12	0.17	0.45	0.0090
												Lower	4.3	0.30	1.60	1.30	1.31	0.58	0.0110
Porterville	Well	0.14	99	256	77	40	41	75	100	688	Navel orange	Upper	4.8	0.46	1.05	0.11	0.06	0.33	0.0180
												Lower	4.5	0.53	0.67	0.70	0.42	0.41	0.0250
Ducor	Well	0.10	48	183	86	31	7	36	100	491	Navel orange	Upper	5.4	0.15	0.79	0.22	0.07	0.38	0.0090
												Lower	6.1	0.16	0.45	0.65	0.22	0.39	0.0075

Source: Harding *et al.* (1958).

plus some leaching rainfall, it should be possible to secure reasonably good citrus performance in some cases with waters of 2,000 ppm of total solids (EC 3.0 mmho/cm, approximately) and fair performance with waters of 3,000 ppm. It is generally considered that total salt levels of about 1,500 ppm and 200 to 250 ppm of chloride represent the upper limit for successful citrus production under most conditions. Even with these, recurring climatic extremes, low leaching rainfall, and soils of poor permeability may rule out their use.

Control Methods.—There is not space here to consider control and management in any detail, but the general principles of dealing with salt and alkali problems in a practical way are fairly well known.

High or fluctuating water tables, especially when they are closer than 4 feet to the soil surface and when they are saline, must be dealt with by appropriate soil-drainage methods. Open ditches, tile drains, or wells (coupled with control of the causes of the high water table, such as canal lining, intercepting drains, and more judicious use of water) are the traditional means of lowering and controlling high water tables.

If a high water table is not a problem or is taken care of by drainage, then the use of sufficient irrigation water to promote periodic root-zone flushing, use of gypsum where too much exchangeable sodium has developed, and use of salt-tolerant varieties of rootstock plus heavier than usual rates of nitrogen fertilizer are the chief means of dealing with the problem of salt and alkali.

Basin or flooding irrigation, use of tensiometers to achieve better control of soil moisture and prevent overirrigation, and periodic soil, leaf, and root analyses to check on salinity conditions and changes will all help in the management problem.

SOIL REACTION: ACIDITY, ALKALINITY, pH

No soil measurements are more meaningful, and yet more difficult to be highly specific about when it comes to relating them to citrus and plant performance generally, than pH and the reserve acidity or alkalinity of soils. Repeated reference has been made in preceding sections to the effects of soil acidity, alkalinity, and pH on the availability, uptake, and toxicity of various elements. There is little need to elaborate here, except to say that within the pH ranges generally encountered in citrus soils the indirect effects or basic soil characteristics (of which pH is an indicator) are important rather than direct effects of hydrogen or hydroxyl ions as such.

Plants have sometimes been classified into acid-loving and nonacid-loving. It is difficult to classify citrus in this regard, since high-performance orchards can be found on soils with pH (paste) values ranging from 4.8 to 7.5 (or on 1:1 suspensions from pH 4.5 to 8.5), and in reserve acidity and alkalinity, from low to high. Citrus is grown on soils with even wider range of pH, but serious problems usually are manifest in soils more alkaline than pH 8.5 or more acid than pH 4.3 (pH on 1:1 soil:water suspensions).

The only generalization possible is that less problems of nutrient deficiency or excess are encountered on soils in the slightly acid range—pH 6.0 to 6.5—than at either higher or lower pH values. In a South African survey of pH in relation to citrus performance, Oberholzer (1944) noted that best growth and yields generally were found on soils of pH 6.0 to 8.5. (He determined pH on a 1:1 suspension with a Beckman glass electrode.)

As far as direct toxic effects of hydrogen and hydroxyl ions on citrus are concerned, these appear unimportant except at very high values. A number of investigators have studied this question. Guest and Chapman (1944) carried out experiments in sand and solution cultures. They found that at pH 2.0 and 11.0 citrus seedlings were killed in a few days. At pH 2.5 and 3.0 the plants did not die, but little growth occurred. Good growth was obtained between pH 4.0 and 8.8 when special precautions were taken to provide ample but not excessive nutrients. Quite a little healthy growth occurred at pH 9.7 at first, but gradually the plants at this pH became light green to yellow and showed both sodium burn and high-sodium levels in the leaves. (Sodium hydroxide had been used to maintain the pH 9.7 level.) Also, some manganese deficiency developed in plants in the pH 8.0, 8.8, and 9.7 range. The fact that plants at pH 9.7 were at first quite healthy suggests that the hydroxyl ion concentration of this pH exerts little direct toxic effect on the plants.

In many years of water-culture experiments with budded orange and lemon trees grown in outdoor cultures maintained at pH values ranging between pH 3.8 and 4.2 (the aim being to maintain a pH of 4.0), the writer has noted healthy root and top growth where sufficient (but not excessive) amounts of other nutrients were present.

Rasmussen and Smith (1959), in water-culture investigations with Pineapple orange seedlings, established evidence of toxicity to roots at pH 4.0. They reported that the roots were brown, stubby, and swollen, in comparison with smooth, white roots grown at pH 6.0. The roots of our water-culture trees at pH 4.0 were generally white, well-branched, proliferated, and not swollen. The writer has frequently noted brown, stubby, and swollen roots of citrus in acid-water cultures where there is a slight excess of copper (e.g., 0.1 ppm of copper). Copper impurities at this concentration can come from bronze-hose bibs or bronze fittings used to dispense water, from distilled water (copper stills), or from chemicals. This or some other toxic impurities or low-nutrient concentrations (direct hydrogen-ion toxicity may be greatly influenced by the amounts and proportions of other ions present, such as calcium, magnesium, and potassium) may account for the observations of Rasmussen and Smith (1959). Girton (1927) noted some root injury and decreased elongation with sour orange seedlings in nutrient solutions of pH 4.0. Best top growth was at pH 6.5, but most root hairs were produced at pH 5.0. Again, the poor growth at pH 4.0 could have been due to copper contamination or low levels of calcium and other nutrients.

In soils, on the other hand, serious problems begin to arise, e.g., excessive solubility of aluminum, manganese, copper, nickel, and other elements in soils of pH 5.0 or less, or deficiencies of phosphorus, calcium, magnesium, and molybdenum. Conversely, as soils become more alkaline, there is decreased solubility of manganese, iron, zinc, boron, copper, and phosphorus.

As stated, no precise limits under soil conditions for top citrus performance can be laid down except to say that, in general, one begins to look for and finds troubles at soil pH values of 4.8 and lower or 8.0 and higher. This usually is due to deficiencies or excesses, imbalances, or soil structure and biological problems.

Martin and Page (1962) grew sweet orange seedlings in treated soils from various citrus orchards. The pH of large quantities of these soils was first adjusted downward by prolonged leaching with hydrochloric acid. The soils became hydrogen-saturated and had pH values as low as 3.3. Excess acid and reaction products were leached out with distilled water. By appropriate additions of calcium carbonate, magnesium carbonate, and

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potassium bicarbonate in various ratios, soils of various pH and exchangeable base content (i.e., calcium, magnesium, and potassium) were produced. These were then potted in 3-gallon jars, and sweet orange seedlings were grown for several months. The data of one of the experiments, showing soil composition, dry weight of plants produced, and leaf and root composition, are reproduced in table 3–54.

Best plant growth was at pH 5.7 to 6.9, with only slightly less growth at pH 4.8. At pH 4.2 and lower, growth was markedly reduced, primarily due to lack of calcium and perhaps aluminum toxicity. It is probable that other potentially toxic metals, e.g., copper, zinc, manganese, and nickel, had been leached out by the prior acid leaching.

Rasmussen and Smith (1957) grew Pineapple orange seedlings in the acid subsoils of three Florida sands. Separate lots of soil were leached with 1*M* sulfuric acid and 1*M* calcium chloride to hydrogen saturate and to calcium saturate each soil, respectively. Excess acid and salt were then leached out with rain water. The hydrogen-saturated soil had a pH of near 4.0, and the calcium-saturated soil, near pH 7.0. These soils were combined in proportions to give pH levels of 4.0, 4.3, 4.6, 5.0, and 6.0. Seedling oranges were then planted. The pots were periodically watered with a nutrient solution containing nitrate and ammonium, adjusted in pH to correspond to the various pH's of the potted soils. After five months of growth, the highest dry weights were at pH 6.0, but in two of the soils almost as good growth was obtained at pH 4.3. Growth was considerably poorer at pH 4.0. It seems probable that the much poorer growth at this low pH was due to lack of sufficient calcium.

In addition to the varying solubility of many inorganic elements in soils, soil pH modifies biological activities in important ways, and these plus the chemical changes, in turn, can influence soil structure and aeration.

Martin, Harding, and Garber (1961) found that the citrus replant problem was somewhat less acute on acid than on neutral and alkaline soils. The precise explanation is unknown, but there is considerable field evidence to support their findings.

Aldrich *et al.* (1955) grew citrus seedlings in potted soils brought to various pH values by the addition of increasing amounts of sulfur. Growth decreased with increasing acidity, but leaf and soil analyses showed that the cause was increased sulfate uptake by the plants (to the point of injury) and the increased soluble salt content of the soils. This work is cited primarily to indicate that soil acidity, and in this instance soil acidification of a naturally neutral to alkaline soil (by means of sulfur additions), may produce byproducts in sufficient amounts to depress plant growth.

Causes and Control of Soil Acidity and Alkalinity.—Soils become naturally acid through

Table 3–54

INFLUENCE OF BASE SATURATION (pH) ON GROWTH AND CHEMICAL COMPOSITION OF SWEET ORANGE SEEDLINGS IN HANFORD SANDY LOAM FROM SANTA ANA CANYON, CALIFORNIA[*]

Exchangeable Cations (Per Cent)					pH of Soil Paste	Growth in Dry Weight of Whole Plant[‡] (Gm)	Composition of Leaves (Per Cent in Dry Matter)				Composition of Leaves (Ppm in Dry Matter)				Composition of Roots (Per Cent in Dry Matter)		
Ca	Mg	K	Apparent H[†]	Al			Ca	Mg	K	P	Zn	Cu	Fe	Mn	Ca	Mg	K
8	6	4	43	39	3.3[§]	8	0.47	0.24	1.9	0.12	30	6.4	75	65	......	......	....
12	7	5	41	35	3.4	12	1.0	0.33	1.6	0.13	24	8.4	52	69	0.14	0.30	1.2
24	8	5	41	22	4.2	66	1.7	0.24	2.1	0.16	21	4.8	56	47	0.18	0.41	1.4
35	10	5	41	9	4.8	92	3.0	0.24	2.0	0.16	23	3.3[‖]	98	49	0.28	0.64	1.4
50	15	5	28	2	5.7	103	4.0	0.26	1.5	0.17	29	2.3[‖]	61	57	0.43	0.77	1.5
80	15	5	0	0	6.9	96	4.2	0.30	1.2	0.16	29	1.5[‖]	62	40	0.68	0.97	1.4
Excess	15	5	0	0	7.4	81	4.4	0.40	1.1	0.18	24	2.9[‖]	45	23	0.70	1.11	1.4

Source: Martin and Page (1962).

[*] Exchange capacity at pH 7 (Ba) = 15.3 meq/100 gm.

[†] By subtracting sum of Ca, Mg, K, and Al from exchange capacity at pH 7.

[‡] LSD at 5% = 6 gm.

[§] Half of plants died.

[‖] Copper deficiency symptoms developed just before plants were harvested.

the prolonged action of rain in dissolving, replacing, and leaching calcium, magnesium, sodium, and to a lesser extent potassium from the silicate minerals and clays which in part came from the original soil-forming materials and form as secondary weathering products during soil formation. These bases are replaced by hydrogen and aluminum ions. Thus, naturally acid soils are generally low in calcium and magnesium, and poor plant growth is frequently due to lack of sufficient amounts of these elements for normal plant development. Also, lack of available phosphorus, potassium, molybdenum, and other elements are frequently encountered in such soils.

Natural soil alkalinity, on the other hand, arises from the weathering of rocks to form carbonates, bicarbonates, chlorides, and sulfates of sodium, calcium, and magnesium, followed by soil enrichment in these through periodic flooding, capillary rise from water tables, and irrigation.

Soils may become acid through continued use of acid-forming fertilizers, such as ammonium sulfate, or applications of sulfur. Conversely, soils may become alkaline through the deposition of salts and carbonates carried by irrigation waters and the application of fertilizers such as calcium nitrate, sodium nitrate, and lime.

Various methods have been worked out to determine the approximate amounts of alkalinizing and acidifying chemicals needed to effect a given change in soils. (A number of such methods are described by Chapman and Pratt, 1961.) The amounts of alkalinizing or acidifying materials required vary greatly, depending primarily on the base-exchange capacity of soils.

Under the intensive irrigation and fertilization program applied to most commercial citrus orchards, soil pH may change either up or down rather quickly and radically. There are many instances, some already referred to in the sections on phosphorus and salinity, in which mineral deficiencies and excesses developed as a result of practices which altered soil pH. Therefore, a periodic check of soil pH (using a 1:1 soil-to-water ratio) in various soil horizons of commercial citrus orchards is advised. Practices should be altered if it appears that soils are becoming more acid than pH 5.5 or more alkaline than pH 8.5.

OXYGEN REQUIREMENTS AND SOIL AERATION

Although not as sensitive to poor aeration as the avocado (Curtis, 1949), citrus is fairly intolerant to "wet feet." Many troubles (such as root rotting, iron chlorosis, and salt injury) are brought on or aggravated by overmoist soil conditions or, perhaps more correctly, lack of sufficient oxygen for root respiration and the secondary soil conditions resulting therefrom.

The classic work of Hoagland and Broyer (1936) and Steward (1935) established that oxygen is indispensable for vital activity in plant roots, and that the energy for nutrient and water absorption comes from the oxidation of carbohydrates in roots.

Considering the importance of aeration to root health of citrus, nutrient and water absorption, resistance to root-decay organisms, and tree performance, this subject has not received deserved attention.

After over thirty years of observations with citrus trees growing under controlled sand- and water-culture conditions and in many types of soils throughout the world, the writer has become convinced that a fair share of citrus disorders or substandard performance stems from inadequate aeration.

In outdoor, continuously aerated water cultures with orange and lemon trees, accidental stoppage of aeration in summer for two days or less will often bring on root rotting. Once started, root rotting is sometimes difficult to arrest. When the drains of sand cultures sometimes become plugged by root growth, a telltale decaying root and peppery smell of the nutrient solution is a sign of trouble. This is frequently followed by iron chlorosis and/or a lackluster appearance of foliage, followed by bronzing, vein chlorosis, and some abcission of leaves.

Citrus trees are more likely to be adversely affected by lack of good aeration in summer than in winter. This probably results from the following: (1) greater root and top growth (increased respiration rate and oxygen need); (2) greater microbiological activity and thus competition for oxygen; (3) less carbohydrate reserve in the plant root and a resulting lowered resistance to soil pathogens; and (4) other factors. Reuther and Crawford (1947) noted much lower levels of oxygen in soil air after an irrigation in summer than in winter. Undoubtedly, this was due to microbial activity.

Prevatt (1959) grew seedlings of rough lemon, sour orange, sweet orange, and Cleopatra mandarin in flooded, sandy soils. The flooding was started at different periods of the year. When

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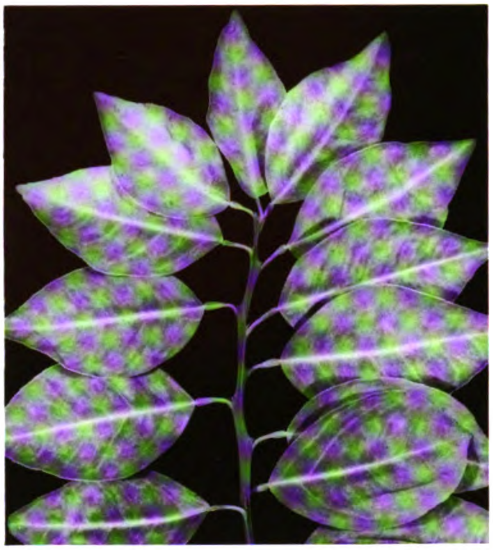

Fig. 3–78. Orange shoot showing veinclearing. This condition is commonly a result of root rotting caused by poor aeration. However, low-nitrogen supply, mechanical injuries to bark or roots, girdling by gophers, calcium deficiency, or senescence can produce a similar leaf condition.

flooding was begun in January (at which time seedlings were making no growth), the plants showed no injury. When flooding was begun in April (after they had started growth), leaf symptoms of water injury appeared after three weeks. Seedlings flooded in June, July, and August showed injury symptoms in two weeks. Seedlings grown in subsoil showed water-injury symptoms sooner than those grown in topsoil when flooding was started in summer months. Carrizo citrange, Troyer citrange, sour orange, and rough lemon seedlings were more tolerant of free water in both limed and unlimed soils than sweet orange, Cleopatra mandarin, and Rusk citrange seedlings. (Prevatt also noted that water extracts of incubated citrus roots and water extracts from waterlogged soil in metal cans contained toxic substances which caused citrus seedlings to wilt and become desiccated.)

Poor soil aeration and resulting poor growth are not only a matter of insufficient oxygen for plant roots, but also carbon dioxide accumulation, the production of traces of other gases (such as hydrogen sulfide, methane, and hydrogen), accumulation of nitrite (in some instances), and increased solubilities of manganese, iron, and other nutrients. It is likely that other secondary products of a toxic nature form. Hence, this subject (like that of pH) requires consideration of both the primary effects of low oxygen as well as the adverse secondary conditions which develop in the wake of low-oxygen levels.

A type of veinclearing in citrus leaves which results from the rotting of roots is shown in figure 3–78. As pointed out in the sections on nitrogen and calcium, this condition can also arise from nitrogen and calcium deficiencies, old age, foot rot, and mechanical injury to the bark of trees, branches, and twigs; hence, it is not specific for waterlogging.

Oxygen requirement and soil aeration have been studied by several techniques: (1) aerating nutrient cultures with gases of various oxygen partial pressures (e.g., oxygen and nitrogen mixtures), in which oxygen is varied from values approaching zero (100 per cent nitrogen) to over 20 per cent, and noting root and top growth, nutrient absorption, carbon dioxide excretion, and metabolic changes in plant roots and tops; (2) passing various oxygen-nitrogen gas mixtures over or into sealed pots of soil in which plants are growing; and (3) sampling and analysis of soil air (for oxygen, carbon dioxide, and other gases) at various depths and seasons of the year, in relation to soil temperature, moisture fluctuations, and soil fertilization.

All of these methods are useful, but they do not measure actual oxygen conditions in the moisture films surrounding the plant root, for, as in all plant root-nutrient relations, adequacy is determined by both a concentration (intensity) and rate of renewal (capacity) factor. It has been repeatedly shown that plants can obtain adequate nitrogen, phosphorus, potassium, and other nutrients from low concentrations provided these are maintained or renewed as fast as they are absorbed by plant roots. The maintenance factors depend on supply, solubility, and diffusion rate. With respect to oxygen, the following are important in determining both concentration in the water film and rate of its renewal: (1) the concentration of oxygen in the soil air; (2) the amount of soil air; (3) the effective diffusion coefficient through the water film surrounding the roots; and (4) the thickness of the water film; and (5) temperature (oxygen solubility decreases as temperature rises).

A measurement of the amount of oxygen which can arrive at a given point per unit time (referred to as oxygen diffusion rate or ODR) is to some extent an integration of the aforementioned concentration, diffusion coefficient, and pathlength factors. The measurement can be made by inserting a platinum electrode and salt bridge leading to an Ag-AgCl cell into a soil, applying a certain electrical potential, and measuring the steady-state current. Current flow at the particular potential chosen is produced by a two-step reduction of oxygen at the platinum-electrode surface involving the transfer of four electrons to each atom of oxygen (O_2) reduced. The current produced under specified conditions then is essentially a measure of the rate of oxygen diffusion (ODR) to the platinum electrode.

Adopting the use of a 25-gauge platinum electrode with an applied voltage of 0.65 volt and readings of current flow after four minutes, Stolzy and Letey (1964) and their associates have made extensive studies. Oxygen diffusion rate (ODR) is conveniently expressed as micrograms of oxygen reduced per square centimeter of electrode surface per minute or, in abbreviated form, μgm cm^{-2} min^{-1}. (Many results have been reported in units of gm cm^{-2} min^{-1} $\times 10^{-8}$. These results can be converted to micrograms by dropping the 10^{-8} and moving the decimal point two places to the left.)

In their preliminary research, plants were grown in sealed, double-walled cylinders of soil (the inside cylinder of plexiglass so as to observe root growth) supplied with various gas mixtures which flowed over the surface of the soil. By inserting the electrode at appropriate ports in the sides of the cylinder and noting root growth through the transparent plexiglass walls in relation to the various gas mixtures, values for oxygen diffusion rate (ODR) at which growth practically ceased have been established for a variety of crops. In the case of citrus seedlings, the critical limiting value for root growth at summer temperatures was about 0.20 μgm oxygen cm^{-2} min^{-1}. At values of 0.31, some root growth occurred. In winter, a higher ODR was required, and it is hypothesized that perhaps in summer the lower ODR requirement is due to some internal movement of oxygen from top to roots.

In studies correlating nutrient uptake with ODR, Stolzy *et al.* (1963) found that leaf concentrations of potassium, phosphorus, calcium, magnesium, iron, manganese, and boron were reduced at ODR's below 0.33 μgm cm^{-2} min^{-1}, compared with an ODR above 0.62. On the other hand, chloride values increased as the ODR dropped.

In further studies of a similar nature, Labanauskas *et al.* (1965) found that uptake of eleven elements was decreased as oxygen supplied over the soil surface decreased. They used sweet orange seedlings of the Bessie variety. Sodium and chloride absorption increased in tops as ODR decreased, and similar results have been noted for manganese.

Stolzy *et al.* (1963) studied the effect of ODR on the citrus nematode *Tylenchulus semipenetrans* and found that this organism was more sensitive to oxygen lack than sweet orange seedling roots.

With respect to root-rotting fungi, Klotz, Stolzy, and DeWolfe (1963) found that *Thielaviopsis basicola* had a higher oxygen requirement (ODR) than *Phytophthora parasitica* and *P. citrophthora*. The *Phytophthora* species were reduced in numbers when the ODR was 0.12 μgm cm^{-2} min^{-1} as compared with 0.22. These preliminary results suggest that the citrus plant is more sensitive to oxygen lack than these organisms.

Many other studies have been made, such as the effect of soil depth (ODR generally decreases with depth, as might be expected), effects of soil compaction, water table, temperature, soil moisture, and air pollutant damage in relation to ODR.

These and many specific literature citations dealing with other crops are briefly mentioned in the summary published in *Advances in Agronomy* by Stolzy and Letey (1964).

Preliminary standards for quite a few crops have already been worked out. The comparative simplicity of the equipment and its portability has made it possible to make measurements in the field, as well as under controlled conditions in the greenhouse and laboratory.

Studies by Stolzy and Letey (1964) and others indicate that with their technique, ODR values of 0.20 μgm cm^{-2} min^{-1} inhibit the root growth of many plants. Values between 0.20 and 0.30 retard root growth. With values greater than 0.40 μgm cm^{-2} min^{-1}, top growth of many plants is not impaired.

Combined with measurements of related or consequential effects in soils (such as other soil gases, solubility of soil constitutents, and activities of microorganisms), a more realistic understanding of the aeration factor as it affects the performance of citrus and other plants is emerging from this newer approach.

Previous data and findings with respect to oxygen requirement and soil aeration are of in-

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terest and are briefly covered here to round out this review.

In short-term, solution-culture studies with sour orange seedlings in nutrient cultures aerated with various oxygen-nitrogen gas mixtures, Girton (1927) found that root growth was completely suppressed when the oxygen content of the gas was 1.2 to 1.5 per cent, as compared with controls receiving normal air. At 5 to 8 per cent of oxygen, root growth was about one half that of the controls. The latter experiment was with solutions held at 25°C, the former at 28° C. The presence of carbon dioxide at 37 to 55 per cent (with oxygen at 16.5 to 20 per cent, the remainder nitrogen) almost completely suppressed root growth.

In experiments with sweet orange seedlings, Cannon (1925) found some root elongation in cultures supplied with gas mixtures with as little as 0.8 per cent oxygen, 5.8 per cent carbon dioxide, and the rest nitrogen. The temperatures were controlled and ranged from 20 to 25°C. Where oxygen was excluded completely, no root growth occurred. In another five-day test, root elongation was 10 mm where the gas mixture contained only 0.5 per cent oxygen, the rest nitrogen, as compared with 20 mm for the cultures receiving ordinary air. In other experiments, the oxygen requirement appeared high. Cannon (1925) concluded from his experiments that the minimum oxygen concentration for the sweet orange is above 1.4 per cent oxygen at a soil temperature of 20°C. Brazilian sour orange seedlings appeared to have a somewhat lower oxygen requirement, ranging from 0.6 to 1.6 per cent. Where the carbon dioxide content of the gas did not exceed 21 per cent, the roots of sweet orange were found to grow when the quantity of oxygen present was less than 2 per cent.

Curtis (1949) grew sweet orange and avocado seedlings in 20-liter Pyrex bottles of nutrient solution supplied with nitrogen-oxygen gas mixtures providing solution concentrations ranging from 0.05 to 28.0 ppm of dissolved oxygen. The work was done in a greenhouse in which solution temperatures ranged from 20 to 25°C.

While root growth of citrus seedlings did not entirely cease during a 10-day aeration period with oxygen at 0.05 to 0.6 ppm, the growth was poor. It was rated as fair at levels of 1 to 2.9 ppm, good at 6.4 to 6.8 ppm, and excellent at 8 to 8.6 ppm. At 16 ppm, growth was only fair, and at 28 to 32 ppm, the roots became stubby. Pure oxygen was used to secure the latter concentration. The avocado was much more sensitive to low oxygen levels, and root growth stopped at 0.05 to 0.06 ppm. The avocado leaves also wilted, whereas there was no leaf wilt of citrus at the lowest oxygen levels. There was root-tip discoloration of citrus roots below 0.7 ppm of oxygen, and after several days the root tips became soft and sloughed off. Injury to citrus roots was noted during the very first day of exposure to low oxygen. Avocado roots showed even greater injury. In spite of the sloughing of new root tips, new citrus roots pushed through the cortex of older roots even at the lowest oxygen concentration.

Reuther and Crawford (1947) studied the fluctuation of oxygen and carbon dioxide in differentially-fertilized field plots with young grapefruit in relation to both frequent (wet) and infrequent irrigation treatments. This work was carried out on a Coachella very fine sandy loam in the Coachella Valley of California. The soil contained considerable free calcium carbonate. Gas sampling wells were established at two soil depths—10 and 20 inches. Determinations were made during all seasons. Trees in the frequently irrigated plots (wet treatments) showed iron chlorosis in winter, but this could not be related to oxygen levels, for in general higher oxygen was found in the soil air in winter than in summer. At no time did the oxygen levels of the soil air in winter fall below 12 per cent. Of course, the oxygen diffusion rate could have been limiting in spite of a good oxygen supply in the pores. One of the striking findings in this investigation was the rather sudden decrease in the oxygen content of soil air, especially at the 10-inch depth shortly after an irrigation. Greatest decline was in a manure-treated plot, where on some occasions the soil air composition changed from 12 to 15 per cent of oxygen before irrigation to less than 1 per cent after irrigation. This low oxygen state held for several days and did not return to the preirrigation levels for almost a week. The sudden drop of oxygen was followed (with a lag period) by an increase in carbon dioxide. These fluctuations after irrigation were much more marked in summer than in winter, reflecting greater plant and microbiological activity.

Already mentioned under the section on iron deficiency is the work of Wallihan *et al.* (1961), who were able to lower the iron content of the leaves of citrus seedlings growing in potted soils, supplied with low-oxygen gas mixtures containing 2 to 6.2 per cent of oxygen. The gas mix-

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tures were passed over the surface of the potted (sealed) soils.

Chapman *et al.* (1965) noted in outdoor water-culture studies with oranges growing under various calcium concentrations that roots under high-calcium conditions could withstand poorer aeration than those growing under low-calcium conditions. Under moderate calcium deficiency, good roots were produced when aeration was at a maximum, but root rotting occurred if aeration was poor.

Improvement of Soil Aeration.—Periodic deficiency of sufficient oxygen for optimum root growth and citrus performance probably occurs to some degree on all but the most sandy, open, or porous soils. It is an especially serious problem where free and rapid downward movement of water is impaired by clay layers, hardpans, plowsoles, and/or perched water tables, where there are prolonged periods of heavy rainfall, or where surface soil structure has been so impaired that water stands on the surface for considerable periods. On many irrigated soils, it is certain that during and immediately after irrigation there are temporary oxygen deficits in local soil zones or areas of marked root development, or in areas where there is considerable organic material and microbial activity is high.

Improvement of soil aeration can be accomplished in many ways. Some are quite simple and inexpensive, while others (such as the installation of tile drains to facilitate movement of excess water out of the soil) are expensive.

Improvement of soil structure, with some improvement in aeration, can be accomplished by the more or less regular use of organic matter (either grown as green manures and turned under or added as manures) or bulky organic materials (such as straws and hays of various kinds). Organic mulches of wood shavings or cotton hulls and periodic mowing of permanent or semipermanent sod cover or weeds are all well-known techniques.

Noncultivation and control of weeds by hand hoeing, mowing, or herbicides is another technique for improving or restoring soil structure in surface layers of soil.

Plowsole which develops from overcultivation will gradually disappear or become less impervious under noncultivation. Compacted layers can be broken up by deep chiseling, and while some roots are destroyed in the process, most experience indicates that no permament harm to the orchard will result.

Deeper clay layers and very heavy soil in which water moves slowly are harder to deal with, but the use of deep-rooted green manure crops, such as pigweed *(Amaranthus retroflexus)*, alfalfa, or other crops, will prove helpful.

Where clay layers or hardpans are not too deep, it is now a common practice before citrus is planted to break these up by deep plowing or chiseling.

Rotational irrigation (in which alternate sections are watered, then allowed to dry to near the wilting point before wetting again) is a common practice in some soils and areas. Tensiometers are especially useful in helping to guide irrigation practices. Many citrus orchards, where periodic iron chlorosis has been a problem, have been strikingly improved by allowing lower soil horizons to dry out before irrigation.

In some areas subject to heavy rainfall for prolonged periods, ridging of soils is a good practice. For example, with certain Florida soils, tree rows are planted on the crests of rather good-sized ridges or "beds." These are constructed so that the height between the ridge crest and bottom is as much as two to three feet. Thus ridge valleys or large furrows separate the tree rows which are used for irrigation during the dry season and as drainage channels to take away excess water during heavy rainy periods.

Vertical mulching with organic matter in trenches is an aid to aeration. In the Ahmednagar district of Bombay presidency of India, Sahasrabuddhe (1927) reported that in a heavy-type soil where orange tree dieback (due probably to poor aeration) was severe, digging of trenches 3 feet wide and 3 feet deep and filling them with broken tile and stone in the bottom and soil plus manure on top greatly reduced the amount of dieback and improved tree color and fruiting as compared with trees which received the same amount of manure applied to the soil surface in a ring around the tree. Best results were obtained with trenches on three sides of the tree, but even on one side of the tree a trench produced substantial improvement. The fertilizer per tree consisted of 20 pounds of farmyard manure, 10 pounds of bone meal, and 3 pounds of oil cake.

Chemical soil treatments to improve aeration have been suggested. Some researchers are of the opinion that ample nitrate fertilizer in the root zone and in roots will provide some oxygen for plant roots during periods of oxygen shortage in the soil. Haas (1937) found in solution cultures that high nitrate could to some degree compensate for lack of aeration. However, Bain and Chapman (1940) grew avocado and grapefruit seedlings in nitrate-treated, waterlogged soils and got no evidence of a beneficial effect. In fact, with the avocado, injury from waterlogging was aggravated by the addition of calcium nitrate. This was probably due to the immediate reduction of some of the nitrate to nitrite, and the latter was toxic to the avocado root.

It is probable that some rootstocks and rootstock-scion combinations are much more tolerant of poor aeration than others. The sour orange, for example, appears to be somewhat more tolerant of low-oxygen conditions than the sweet orange. Much more information is needed in this field than now exists.

From the preceding discussion of oxygen requirement and review of existing information, it is quite evident that citrus is reasonably tolerant to low-oxygen conditions and that complete cessation of root growth and actual root injury may not occur until levels of oxygen in soil moisture films are at levels below 0.5 ppm of oxygen. However, best root growth occurs only when oxygen is somewhere near its maximum solubility (8 ppm at 25°C).

The investigations of Stolzy and Letey (1964), using their oxygen diffusion rate (ODR) technique for determining oxygen status, have provided an excellent tool for studying this problem much more rapidly and thoroughly than has been possible hitherto. From a practical point of view, it is especially important to find out whether, or the extent to which, top performance is impaired by temporary periods of poor aeration, the effects of variable nutrition on oxygen need, rootstock tolerance and effects, and relations to compacted subsoil layers, recurring climatic extremes, and other problems.

Knowledge in these areas may point the way to better means of coping with soil aeration problems under a wide range of soil conditions.

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CHAPTER 4

Seed Reproduction: Development of Gametes and Embryos

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SEED REPRODUCTION of citrus is a continuous process that begins in the flower and ends with maturity of the fruit (fig. 4–1). All or nearly all of the flowers of some sterile varieties, including certain triploids and also certain defective flowers, such as those with abortive pistils (p. 314), fall from the tree with little if any growth after the petals separate. In fertile varieties, flowers or ovaries may fall at any time from the unopened-bud stage up to the time of definite fruit "setting," some ten or twelve weeks after the flowers open. By then, the normal removal of surplus ovaries is complete; except for rare accidents, the remaining fruits stay firmly attached to the tree until maturity. The setting of fruits depends on complex physiological conditions that relate to the supply of water, food materials, and hormones, and which therefore are much affected by competition among flowers and young fruits. In many varieties, the reproductive processes of pollination, fecundation, and embryo formation are of primary importance for fruit setting.

DEVELOPMENT OF EMBRYO SAC AND FEMALE GAMETE

The general structure of the pistil of *Citrus* has been described in Chapter 1, but a more detailed account of its reproductive apparatus is presented here.

Carpels and Placenta

The pistil of *Citrus* is composed of about ten units, the carpels, each somewhat resembling a bean pod in structural plan, which are joined to each other and to a central axis to form a compound pistil. In the mature flower, the pistil is composed of the ovary (the young fruit) at the base, the stigma (apical knob), and usually a slender style between. At a very early stage (fig. 4–2, *C*), the pistil is not closed at the top, but consists of a circular wall or ring and a protuberance within the ring (Osawa, 1912). This wall is composed of the joined carpels, which according to Ford (1942) arise as a whorl of crescent-shaped primordia. The carpels grow upward and their margins project inward to meet the central protuberance (fig. 4–2, *D*). At the same time, the central protuberance grows upward to produce the axis or core of the fruit, and unites with the carpel margins. Each carpel thus encloses a locule of the ovary (fig. 4–2, *E*).

At the inner angle of each locule is developed the placenta, the region of thickened tissue which bears the ovules. Biermann (1896) and Ford (1942) stated that the ovules arise from the carpellary margins, but Osawa (1912) considered the placenta to be an outgrowth from the axis.

A stylar canal, lined with epidermal cells

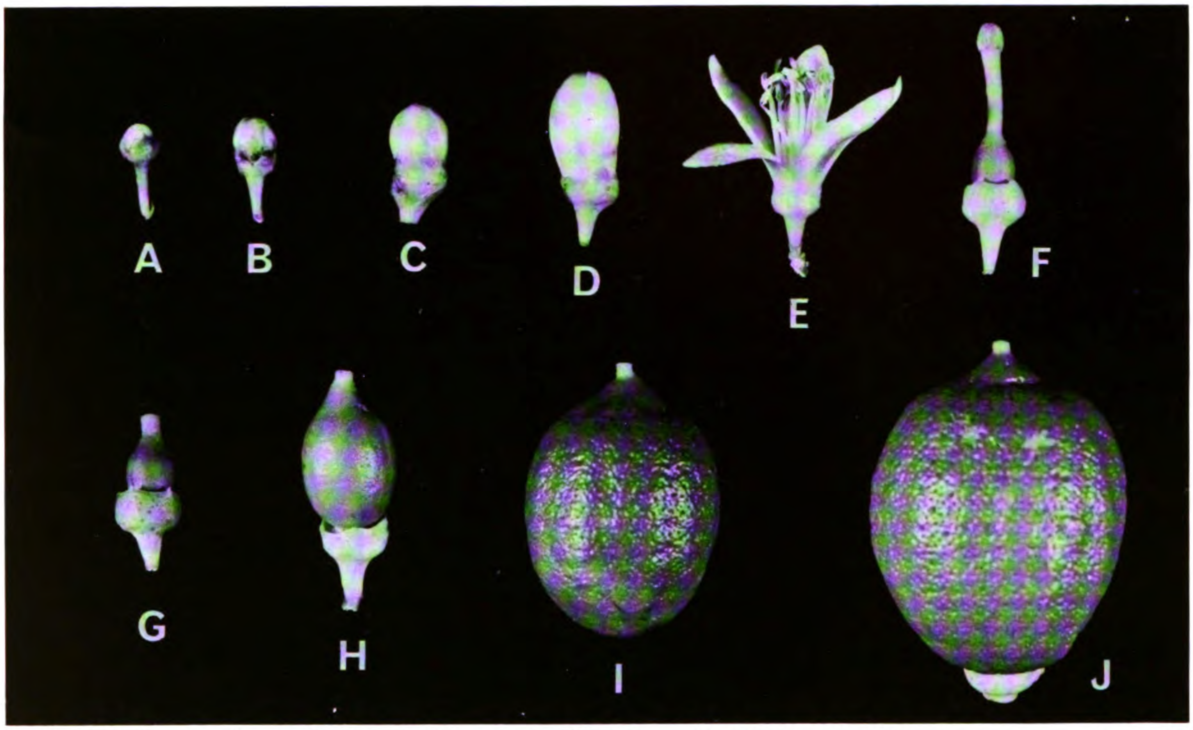

Fig. 4–1. Stages of reproduction in *Citrus limon* (L.) Burm. f. *A*, bud probably having pollen mother cells before prophase of first meiotic division; *B*, bud during meiosis in anthers; *C*, bud at stage of young pollen grains; *D*, bud nearly full-grown; *E*, flower at time of pollination; *F*, pistil just after fall of petals and stamens (about six days after pollination); *G*, ovary just after abscission of style (about 10 or 15 days after anthesis); *H* and *I*, enlarged ovaries of the size reached at time of normal fruit drop; *J*, young fruit decidedly set.

(Tillson and Bamford, 1938; Ford, 1942; Banerji, 1954), extends from the placenta of each carpel to a narrow slit on the surface of the stigma. Through these canals, the pollen tubes grow downward to the ovules. However, Banerji (1954) stated that pollen-tube growth is mainly intercellular and not through the stylar canals.

Ovules

While the flower is still very small, the ovule arises as an outgrowth of the placenta; this outgrowth involves several cell layers. The ovule soon begins to grow upward, and finally takes the typical anatropous (inverted) form, with the micropyle facing the axis of the ovary. The mature ovule consists of the funiculus (stalk), a central mass of cells (the nucellus), the embryo sac within the nucellus, and the two integuments surrounding the nucellus; at the apex of the nucellus is an opening through the integuments, the micropyle. In the anatropous ovule, the end away from the placenta is the chalaza region.

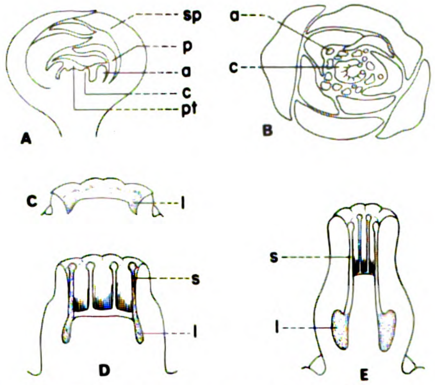

Fig. 4–2. Diagrammatic sections of young flower buds. *A*, longitudinal section at very early stage; *B*, cross-section of older bud, showing locules of ovary enclosed, but center of pistil still open above ovary; *C*, *D*, and *E*, development of locules and stylar canals. Key to symbols: *a*, anther; *c*, carpellary ring; *l*, locule; *p*, petal; *pt*, axis of pistil; *s*, stylar canal; *sp*, sepal. (*A* and *B*, Washington navel orange, after Osawa, 1912; *C*, *D*, and *E*, Eureka lemon, after Ford, 1942.)

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Archesporium and Embryo-Sac Mother Cell

Before the integuments develop, one cell in the second layer, near the apex of the nucellus, is distinguishable by its greater size and its larger nucleus (fig. 4–3, *A*). This is the archesporial cell. The archesporial cell soon divides once; the outer of the two daughter cells is the tapetal cell and the inner one is the embryo-sac mother cell, or megasporocyte (fig. 4–3, *B*). The tapetal cell divides, and from it are developed eight layers of cells; by this time, therefore, the embryo-sac mother cell is deeply buried in tissue near the center of the nucellus. Figure 4–3, *C* shows the development of the tapetal cells and also that of the integuments partly completed. Occasionally more than one embryo-sac mother cell is formed in an ovule. Bacchi (1943) and Banerji (1954) reported more than one normal embryo sac in a single ovule.

Meiosis and Megaspores

The embryo-sac mother cell grows to several times its original size and becomes elongated (fig. 4–3, *D*). The chromosomes of its nucleus meanwhile pair during the prophase stage of the first division. This division occurs before the ovule is fully developed (Bacchi, 1943). By metaphase of the first division, the nuclear membrane disappears. Each chromosome pair separates during anaphase and telophase, forming two groups of chromosomes. Following a short interphase, the second division splits each chromosome in two, resulting in four equal groups of chromosomes. After the second division, cell walls are formed, producing four cells in a row that extends longitudinally in the nucellus (fig. 4–3, *G*). These four cells are the megaspores.

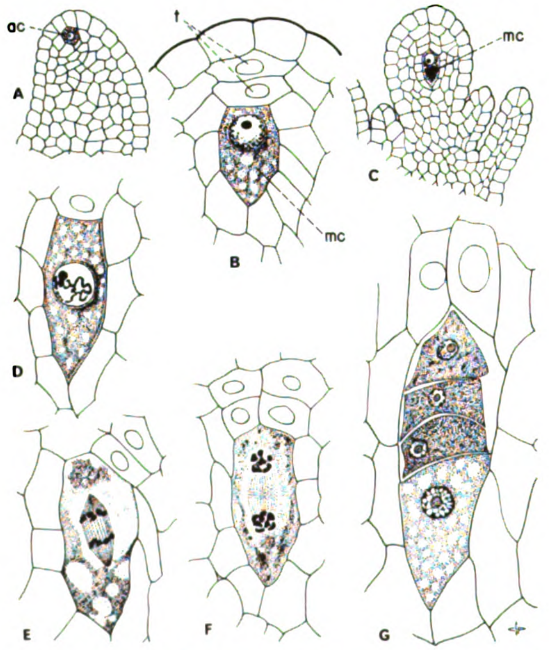

Fig. 4–3. Development of the megaspore (*F*, *Poncirus trifoliata* (L.) Raf.; rest, satsuma mandarin). *A*, very young ovule with hypodermal archesporial cell; *B*, megasporocyte and tapetal cells; *C*, portion of older ovule, showing integuments and megasporocyte; *D-F*, megasporocyte (embryo-sac mother cell) during meiosis. *G*, row of four megaspores, resulting from meiosis (*ac*, archesporial cell; *mc*, megasporocyte; *t*, tapetal cells). (Redrawn from Osawa, 1912.)

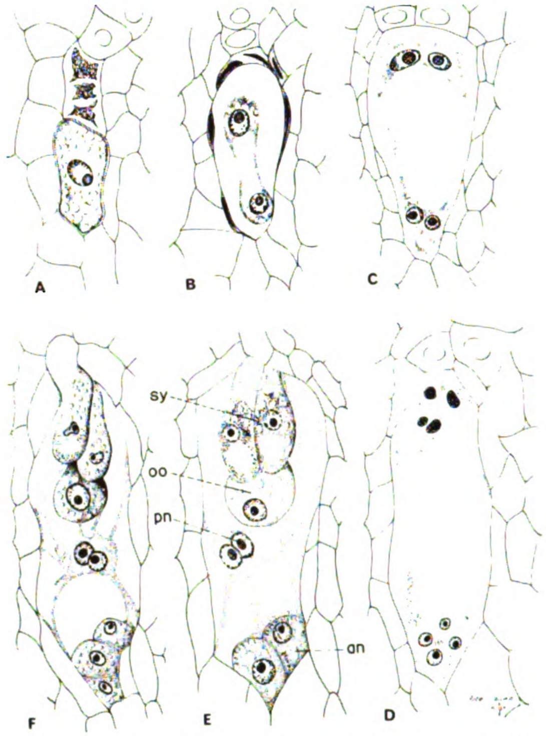

Fig. 4–4. Development of the embryo sac (*B*, *Poncirus trifoliata;* *F*, Washington navel orange; rest, satsuma mandarin). *A*, four megaspores, top three degenerating and lowest enlarging; *B-F*, embryo sac, with two nuclei (*B*), with four nuclei (*C*), with eight nuclei (*D*), and mature (*E*, *F*). Key to symbols: *an*, antipodal cells; *oo*, egg cell; *pn*, polar nuclei; *sy*, synergids. (Redrawn from Osawa, 1912.)

Embryo Sac and Egg Cell

Only the lowermost megaspore develops further; the remaining cells degenerate (fig. 4-4, *A*). The lowest cell grows longitudinally and occupies the space vacated by the others. This cell, the functional megaspore, develops further to

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form the embryo sac. It enlarges greatly, but its cytoplasm does not increase correspondingly; large vacuoles appear, and when the embryo sac is mature they occupy the largest part of the volume within the cell wall (fig. 4–4).

As the megaspore grows, its nucleus divides, and the two daughter nuclei go to the opposite ends of the embryo sac (fig. 4–4, *B*). Each nucleus divides again, so that there are four nuclei in the embryo sac (fig. 4–4, *C*). Each of these four nuclei divides once more, thus producing eight nuclei; the embryo sac now contains four nuclei near each end (fig. 4–4, *D*). Three nuclei remain near the basal (chalazal) end, where they organize the three antipodal cells (fig. 4–4, *E*). In the micropylar end, three complete cells are also arranged. One of these is the egg cell, and the other two are the synergids, which perhaps facilitate the process of fecundation of the egg cell (fig. 4-4, *E*). At this stage, the egg is mature and ready for fecundation.

The two remaining nuclei, one near each end of the embryo sac, are the polar nuclei. These move toward the middle of the sac (fig. 4–4, *F*) and supposedly fuse to form the endosperm nucleus. (See p. 300).

The corresponding stages of development are attained later in the ovary than in the anthers of the same flower, so that the microspores have begun to develop into pollen grains before the megasporocyte has passed the prophase of the first division (Osawa, 1912). At the time of the opening of the flower (fig. 4–1), the embryo sac is usually in the eight-nucleate stage, but may be in the one-, two-, or four-nucleate stage (Bacchi, 1943).

DEVELOPMENT OF POLLEN AND MALE GAMETES

Development and Opening of the Anther

A general description of the anthers has been given in Chapter 1. Since phylogenetically they are presumed to be modified leaves and bear the microspores, the stamens are microsporophylls. Each of the four lobes of the anther normally develops a microsporangium or enclosure in which microspores form and develop into pollen grains. Apparently the two microsporangia in each half of the anther usually coalesce, so that in the mature anther the pollen is held in two pollen sacs or anther locules; according to Osawa's drawings (Osawa, 1912), however, the microsporangia of the satsuma mandarin often remain separate, at least when imperfectly developed. Each half of the anther dehisces by a longitudinal split between the lobes, about the time of the separation of the petals; the epidermis dries out and rolls back the anther wall, exposing the enclosed pollen as sticky, yellow dust.

Archesporium and Pollen Mother Cells

At an early stage in development of the anther, the archesporial cells are recognizable by their larger nuclei and different staining; as in the megasporangium, these cells are derived from the hypodermal layer (Banerji, 1954). According to Banerji (1954), the archesporial cells divide periclinally to produce an outer layer of parietal cells and an inner layer of sporogenous cells. Additional divisions of the parietal cells produce four cell layers. The innermost layer forms the tapetum, and the others with the epidermis form the anther wall.

The tapetum is mainly one-layered but may be two- or three-layered (Banerji, 1954). It surrounds the slender cylinder of pollen mother cells or microsporocytes (fig. 4–5), which are formed by successive divisions of the primary sporogenous cells. Before the mother cells reach mid-prophase, the nucleus of each tapetal cell divides once; later more divisions occur, so that the tapetal cells become binucleate or irregularly multinucleate. While the development of the pollen grains is in progress, the tapetal cells disintegrate, probably supplying food materials to the pollen.

Meiosis and Microspores in Diploids

Virtually all cultivated forms of *Citrus, Fortunella,* and *Poncirus* are diploid, and the mono-

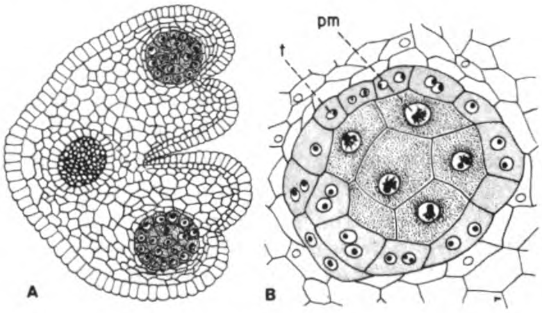

Fig. 4–5. Development of the microsporocytes in satsuma. *A*, cross-section of anther, showing sporogenous cells in two lobes only; *B*, a microsporangium in cross-section (*pm*, microsporocytes; *t*, tapetal cells). (Redrawn from Osawa, 1912.)

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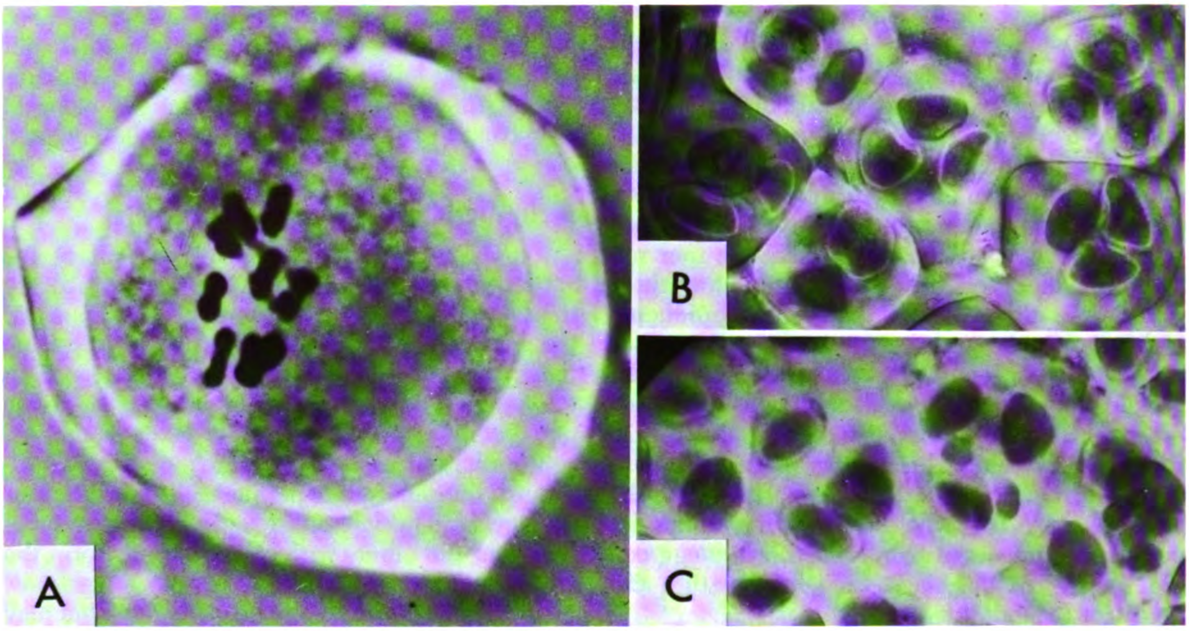

Fig. 4–6. Meiosis in microsporocyte and formation of microspores. *A*, pre-metaphase in Eureka lemon, showing nine bivalents; *B*, sporads of diploid Eureka lemon showing regular development; *C*, sporads of a triploid hybrid showing irregular development, resulting in extra microspores.

ploid number of chromosomes in these genera is nine (fig. 4–6). (See Longley, 1925; Frost, 1925*a*; Nakamura, 1934; Ruggieri, 1935; Miedzyrzecki, 1936; Lapin, 1937; Kandelaki, 1938; Bacchi, 1940; Yarnell, 1940; Nakamura, 1941, 1942; Krug, 1943; Nakamura, 1943; Chen, 1944; Singh and Shah, 1950; Rhandhawa and Choudhury, 1960; Ozsan, 1961; Naithani and Raghuvanshi, 1962; Raghuvanshi, 1962*a*.) Diploidy ($n = x = 9$) occurs also in *Severinia, Triphasia, Citropsis, Aeglopsis* (Longley, 1925); *Feronia, Murraya* (Toxopeus, 1933; Pathak, *et al.*, 1949); *Afraegle, Atlantia, Clausena, Microcitrus* (Krug, 1943); *Eremocitrus, Micromelum* (Yarnell, 1940).

Before their first division, the pollen mother cells are distinguishable from the tapetal cells which surround them by their large size and possession of only one nucleus, and by differences in staining (fig. 4–5, *B*). During the late prophase of the first division, the pollen mother cells round off. The original mother-cell wall persists about in its original form until it breaks down at the end of the sporad stage, whereas the cytoplasm develops a new rounded wall, usually well inside the original wall (fig. 4–6).

In the pollen mother cells of *Citrus,* as in the embryo-sac mother cells, there has been little study of the prophase of the first division. The processes must usually proceed normally, however, since in later stages the diploid varieties studied generally have nine bivalent chromosomes (fig. 4–6).

At diakinesis the chromosomes are widely scattered in the nucleus (fig. 4–7). The nuclear membrane disappears prior to metaphase, and at metaphase the nine-paired chromosomes become oriented at the equator of the spindle, which has meantime formed, and parallel with its axis. Yarnell (1938) and Nakamura (1939) reported secondary associations of the chromosomes during the beginning of first metaphase. Naithani and Raghuvanshi (1958) and Raghuvanshi (1962*b*) reported multivalents, inversions, and univalents in four varieties. Pereau-Leroy (1950) also reported univalents in the lemon variety Sanguin Panache. Iwamasa (1966) reported a translocation in Valencia, an inversion in Mexican lime, and asynapsis in Mukaku Yuzu.

At anaphase I, the nine bivalents usually separate normally, resulting in two daughter nuclei. Following a short interkinesis stage, the membranes of the daughter nuclei disappear and the second division usually proceeds normally. Usually there are two sets of nine chromosomes each at the metaphase of second division (fig. 4–7, *E*), but irregular distributions have been found, indi-

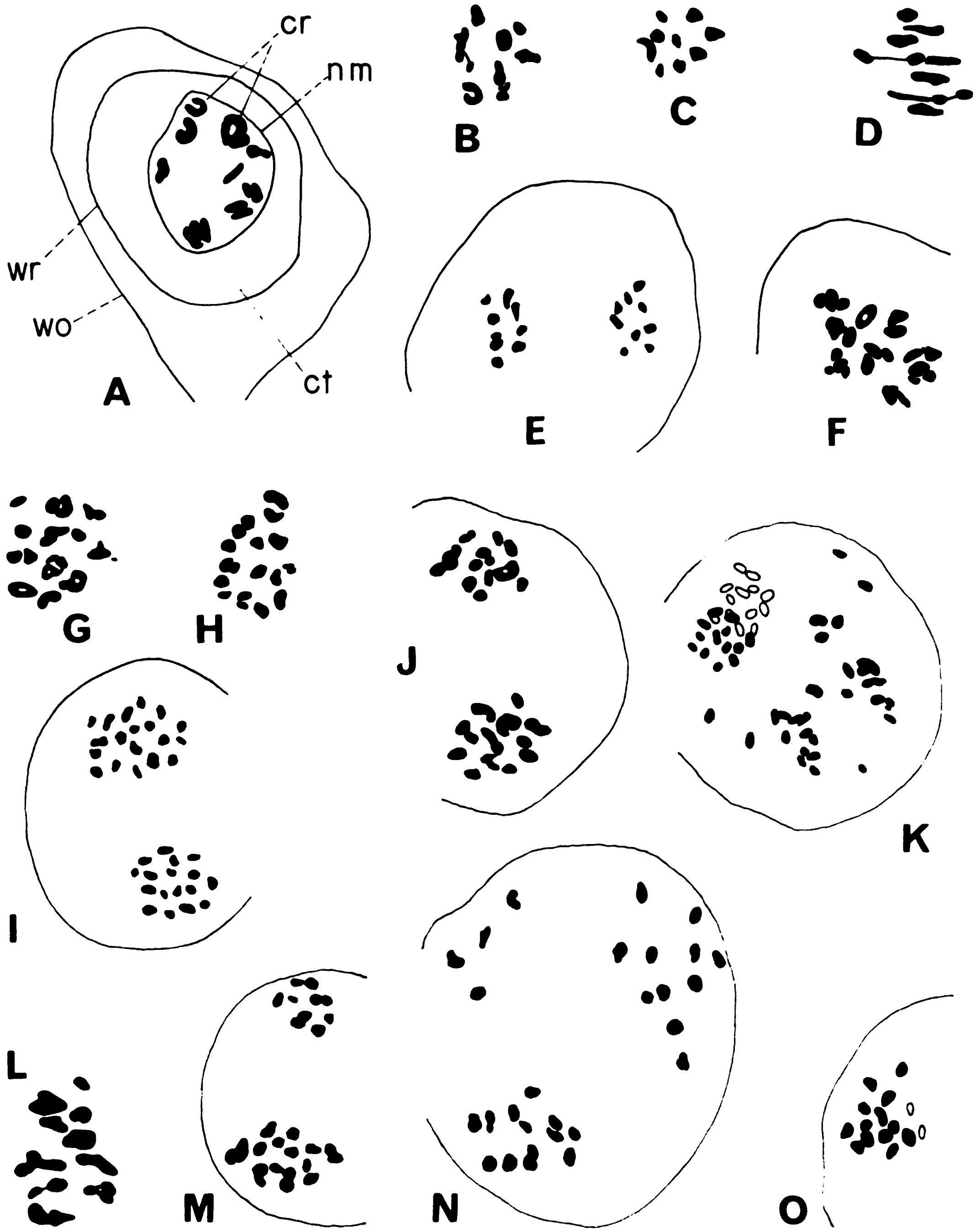

Fig. 4–7. Meiosis in microsporocyte; *A*-*E*, diploids; *F*-*K*, tetraploids; *L*-*O*, triploids. *A*, late diakinesis, eight bivalent and two univalent chromosomes; *B*, slightly before metaphase I, nine bivalents; *C*, metaphase I, polar view; *D*, beginning of anaphase, lateral view; *E*, metaphase II, polar view; *F*, late diakinesis I, presumably nine quadrivalents (compare with *A*); *G*, $6_{IV} + 1_{III} + 4_{II} + 1_{I}$; *H*, largely bivalent; *I*, metaphase II, 19_{I} and 17_{I}; *J*, metaphase II, some bivalents present; *K*, anaphase II, left groups both thirteen, right groups not countable but several strays present; *L*, late diakinesis I, $8_{III} + 1_{II} + 1_{I}$ (compare with *A* and *F*); *M*, metaphase II, $10_{I} + 17_{I}$; *N*, metaphase II, one plate, 13_{I} + 2 halves of univalents. Key to symbols: *cr*, chromosomes; *ct*, cytoplasm; *nm*, nuclear membrane; *wo*, original wall of microsporocyte; *wr*, secondary wall.

cating irregular separation in the first division. Chen (1944) and Banerji (1954) also reported secondary association of chromosomes. At the end of the second division, the four sets of nine chromosomes each organize four nuclei within the rounded wall of the mother cell, producing the four-nucleate stage.

Walls form between the four nuclei, and the four new cells (microspores or young pollen grains) separate and round off within the original wall of the pollen mother cell. This is the pollen-tetrad, microspore-tetrad, or quartet stage. Since more than four microspores may be formed by one mother cell (occasionally in diploids, and abundantly in most polyploids), these terms are often literally inappropriate, and therefore it seems preferable to use the terms sporad and microsporad (Webber, 1933).

The chromosomes are small in bulk. At maximum condensation in the first division, the length is commonly near two microns (1/12,000 of an inch) or less (fig. 4–6), but just before separation in early anaphase I, they may be stretched to a considerably greater length. Yarnell (1937), Kandelaki (1938), and Sharma and Bal (1957) related these differences to various species.

Meiosis in Polyploids

The presence of extra genomes in triploid and tetraploid forms of citrus causes certain characteristics from related diploids in tree and fruit (see chap. 5).

In triploids, chromosome reduction cannot be regular, since there are three somatic chromosomes of each kind and the first division separates the chromosomes of each kind into two groups.

Although Russo and Torrisi (1951*b*) reported allotetraploids as well as autotetraploids, most, if not all, tetraploid forms of *Citrus* are basically autotetraploids in regard to chromosome homology. Therefore, meiosis in tetraploid *Citrus* is irregular, due to multivalent conjugation.

Triploid forms of *Citrus* seem to originate from diploids only as gametic seedlings; tetraploids originate either as nucellar seedlings or in stem growing points (see chap. 5).

Meiosis in Tetraploid Forms

Pollen mother cells and sporads from tetraploid forms of twelve varieties belonging to four species of *Citrus,* have been examined at the University of California Citrus Research Center, Riverside. With some varieties at least, the pollen mother cells of tetraploids degenerate before the reduction division much more often than do those of corresponding diploids. With some varieties, complete counts were difficult to obtain; however, all forms classed as tetraploid had a chromosome number of thirty-six, or at least near thirty-six. Lapin (1937), Furusato (1952), and Russo and Torrisi (1951*a*, 1951*b*) have also reported plants with tetraploid chromosome counts in several varieties.

At and near metaphase I, there is much variation in chromosome conjugation. In especially open figures, at a stage when the chromosomes are not yet oriented at the equator of the spindle, occasional pollen mother cells clearly have nine compound chromosomes (fig. 4–7, *F*). These quadrivalents often look like ordinary bivalents joined by somewhat slender connections, but they also take other forms. Often these are several separate bivalents, and sometimes apparently unconnected bivalents predominate (fig. 4–7, *H*). Trivalent and univalent chromosomes are also common (fig. 4–7, *G*).

At metaphase II (Frost, 1925*b*), the numbers are very irregular, metaphase groups of eighteen univalents being in the minority. In some forms at least, unquestionable bivalents are common (fig. 4–7, *J*). Stray chromosomes, which lagged at anaphase I, are common in the second division (fig. 4–7, *K*).

The sporad stage usually indicates much irregularity of chromosome reduction (fig. 4–6). In most tetraploid varieties studied, from one third to one half of the sporads counted have shown more than the normal four microspores, often six or seven. In tetraploid Owari satsuma, however, only about one sixth to one fourth of the sporads had extra microspores.

Where extra microspores are common, possibly extra nuclei are also present in some microspores. Presumably, also, many irregular chromosome assortments do not lead to the production of extra nuclei. Therefore, tetraploids usually produce few pollen grains whose chromosome complement is completely normal. However, tetraploids have been successfully used as pollen parents in the production of triploid hybrids (see chap. 5).

Meiosis in Triploid Hybrids

Longley (1926) studied the chromosomes in the pollen mother cells of a triploid hybrid from a cross of limequat by kumquat [(*Citrus auranti-*

folia (Christm.) Swing. × *Fortunella japonica* (Thunb.) Swing.) × *F. hindsii* (Champ.) Swing.], the last mentioned species having thirty-six chromosomes. Apparently trivalent chromosomes were common; sometimes several univalents were present.

Frost (1927, 1929, 1930, and unpublished) examined chromosomes from about twenty triploid or approximately triploid hybrids from diploid parents. Sporad counts were also made for triploid trees. As in Longley's (1926) material, first-metaphase and pre-metaphase figures were seldom completely countable in the sense of positive determination of the number of univalent elements present, but they were diagnostic of triploidy or approximate triploidy because of the frequent occurrence of conspicuous trivalents. The three univalent elements were very often joined end-to-end. Other common shapes observed were the **T**, the **V**, the **Y**, and the ring and rod (fig. 4–7). Trivalents usually predominated, but bivalents and univalents were common, often being more numerous than shown in the figures.

Luss (1935), Lapin (1937), Yarnell (1939), and Russo and Torrisi (1951*b*) reported twenty-seven chromosomes in the root tips of various seedling forms.

In the second division, the number of chromosomes in each metaphase group is highly variable, but usually exceeds the normal nine (fig. 4–7, *M*). Some of the chromosomes are often astray, scattered through the cell or in groups (fig. 4–7, *N*). Occasionally, under favorable conditions permitting a complete count, the total number of univalent elements found was twenty-seven.

In triploids, as in tetraploids, the determination of chromosome number at anaphase I and metaphase II may be complicated by the presence of persistent bivalents.

From the number and size of the chromosomes at metaphase II, it appears that the separate univalents of metaphase I must often divide in anaphase I, presumably by precocious separation of the chromatids (fig. 4–7, *O*). This is indicated also by the occurrence at anaphase II of single equatorial laggards equal in size to the anaphase chromosomes.

The sporad stage (fig. 4–6) shows much irregularity of cell division, although in some triploids, as in Longley's (1926) material, a large majority of the sporads have no extra microspores. As with tetraploids, different masses of sporads from the same bud often differ surprisingly in the number of extra microspores; such variations seem to be due to variation in the number of nuclei.

Aneuploids

Luss (1935), Krug and Bacchi (1943), Bacchi (1944), Ohta and Furusato (1957*b*), and Sharma and Bal (1957) reported as many as three chromosomes more than the diploid number in somatic cells. Meiotic studies on aneuploids have not been reported.

Pollen Grain and Sperm Cells (Male Gametes)

Development follows the usual course for angiosperm pollen. The microspore enlarges, and develops two heavy coats, exine and intine. Before the anther dehisces, the nucleus divides, forming the vegetative or tube nucleus and the generative nucleus. J. L. Brewbaker (unpublished) reported the pollen to be binucleate. Banerji (1954), however, reported the pollen of *C. paradisi* Macf. was trinucleate.

Normal anthers are bright yellow when mature, owing to the pollen which they contain. In varieties with very defective pollen development, the anther color may be considerably lighter, whereas anthers containing no pollen are pale cream or white and usually do not dehisce (see p. 315).

Herrero-Egaña and Acerete (1935) made a detailed study of the pollens of varieties belonging to five of the most important species of *Citrus*. They found that lemon pollen was readily distinguished from that of other species by its morphological characteristics. Pereau-Leroy (1950) reported differences in the average diameter of the pollen grains of five species and two hybrids. Nakamura (1943) listed pollen diameters for seven lemon varieties. All but the Villafranca are within the range (34 to 36 microns) given by Pereau-Leroy for *C. limon* (L.) Burm. f. Nair and Mehra (1962) reported distinguishing differences of the colpi and the lumina of the surface reticulations among the five species, *Citrus aurantifolia, C. sinensis* (L.) Osbeck., *C. grandis* (L.) Osbeck., *C. limon*, and *C. aurantium* L.

POLLINATION AND FECUNDATION

Pollination consists of the transfer of pollen from the anthers to the stigma. Fecundation (or fertilization) consists of the fusion of a sperm nucleus with an egg nucleus. A necessary preliminary to fecundation is growth of the pollen tube, from the pollen grain on the stigma to the embryo sac in the ovule.

Selfing and Crossing

Pollination of a pistil by pollen from stamens on the same plant is self-pollination, and fecundation of an egg cell by a sperm cell of the same plant is self-fecundation; both processes are often called selfing. When a plant is divided by asexual propagation to produce a clone, or clonal variety, all the trees of this variety are genetically identical (unless changes are produced by bud variation); pollination or fecundation between such identical trees is self-pollination or self-fecundation of a clone, and is equivalent to selfing of an individual tree.

Cross-pollination and cross-fecundation involve the transfer of pollen from one plant or clonal variety to another. Obviously, there is a genetic distinction between cross-pollination and self-pollination only where the two cross-pollinating plants are in some degree unlike genetically.

Agencies of Pollination

Natural self-pollination can be brought about in *Citrus* by contact of anthers with the stigma, by pollen falling or being blown against the stigma, or by transfer of pollen by insects. The pollen is of the sticky, adherent type characteristic of entomophilous (insect-pollinated) plants, and wind is therefore usually a minor factor in pollination. Four characteristics of the flowers make them attractive to insects: the conspicuous corolla, the strong perfume, the pollen, and the abundant nectar (Webber, 1930). Thrips are present in the flowers in great abundance, some apparently feeding on the pollen. Honey bees and other insects work the flowers for both nectar and pollen. Mites are sometimes found in the flowers and may occasionally inadvertently carry pollen (Uphof, 1934). Natural cross-pollination is undoubtedly accomplished mainly by bees.

In many citrus varieties with functional pollen, fruits with seeds frequently set on branches or trees that have been caged to exclude insects. Thus, in some varieties at least, self-pollination occurs without the agency of insects. Toxopeus (1931) found certain species and varieties to be somewhat proterandous (stamens maturing before the stigma), whereas in other forms the stamens and stigma were found to mature at about the same time. In the former, there is a greater tendency for abundant self-pollination in the absence of insects, because the anthers begin to shed their pollen while they are still pressed against the stigma in the unopened or opening flower. However, other factors may still prevent self-fecundation (see p. 312). The relative length of pistil and stamens may also affect the opportunity for self-pollination (Uphof, 1934).

Relation of Pollination to Setting of Fruit

The significance of pollination for fruit production differs greatly in different varieties. The principal factors concerned are the following: the amount of functional pollen, the facilities for pollination, the relation of the pollen to the setting of seed, and the ability of some varieties to produce seedless (parthenocarpic) fruits either with or without pollination. The main evidence on the factors affecting seed production is discussed under the section headings of "Polyembryony" and "Sterility."

Parthenocarpy (Seedless Fruits)

The production of fruits without seeds is parthenocarpy. The setting of seedless fruits without any external stimulation is autonomic parthenocarpy. The ordinary varieties of navel orange, satsuma mandarin, Tahiti lime, and certain other citrus varieties regularly produce parthenocarpic fruits, and many varieties usually seedy are more or less capable of parthenocarpy. In varieties normally more or less seedy, the tendency to parthenocarpy is highly variable. Ikeda (1904–06) and Nagai and Tanikawa (1928) found that when pollination was experimentally prevented, some varieties set no fruits, and other normally seedy varieties produced seedless fruits, usually setting much less abundantly, however, than when pollinated. Many lemon varieties and Marsh grapefruit readily produce seedless fruits. Valencia orange sets some seedless fruits when pollen is excluded (Wong, 1939). Wilking mandarin set no fruit when pollen was excluded in trials at the University of California Citrus Research Center, Riverside.

In certain plants, the formation of seedless fruits may be induced by pollen which, in the particular combination at least, is incapable of producing fecundation or the setting of seed. Seedless fruits sometimes develop following self-pollination of self-incompatible citrus varieties. Vituskina (1953) reported the set of lemon fruit after pollination with lily pollen.

In citrus, growth-regulating compounds have not generally been effective in setting parthenocarpic fruits. Furusato and Ohta (1957) and Furusato and Suzuki (1955) reported a few seedless fruits in *Citrus natsudaidai* Hayata after treatment with 2,4,5,-trichlorophenoxyacetic acid (2,4,5-T). Gibberellin set seedless fruits on Clementine mandarin (Soost, 1958) and Orlando tangelo (Krezdorn and Cohen, 1962).

Sokol'skaja (1940) and Plaut (1947) reported that seedless fruits were induced by breaking off the pistil prior to anthesis. The seedless Valencia fruits reported by Wong (1939) were also set from flowers in which the pistils had been removed. The stimulation of the formation of plant-growth regulators or the removal of inhibitors by the removal of the pistil are interesting conjectures.

Pollination and Fruit Yields

Although the tendency to parthenocarpy is remarkably prevalent among the citrus fruits, the horticulturally successful parthenocarpic varieties seem to be exceptional in their high ability to set fruits without seeds. If a variety incapable of producing seeds is to be horticulturally successful, it must be highly parthenocarpic; probably many seedless varieties have arisen which were worthless because they were not sufficiently parthenocarpic. In decidedly seedy varieties, on the contrary, the degree of capacity for parthenocarpy is unimportant and ordinarily unnoticed.

There is some reason to believe that seedlessness is rather generally a handicap to the setting of fruit. Even the Washington navel orange is probably limited in yield by its seedlessness. High temperatures during or immediately after fruit set can cause severe shedding of fruit in Washington navel orange. Seedy varieties are less severely affected. Coit and Hodgson (1919) also found that abscission of the flowers occurs more readily in Washington navel than in several less sterile varieties. It is probable that the low productivity of many triploids is due to both ovule and pollen sterility (see chap. 5).

Cross-pollination cannot have much effect on yields unless it occurs on a wide scale. Any effect of pollination is likely to be of little importance in large solid plantings of varieties that have no good pollen of their own, since only trees not far apart are ordinarily likely to interpollinate extensively. Shamel's (1918) observations indicated that there was little pollination in the Washington navel orange plantings which he studied, since seeds were found in only about ten out of more than 25,000 fruits of performance-record trees examined, though artificial cross-pollination of this variety very often produces a few seeds. Webber (1930) concluded that insect pollination is not likely to have any important effect on the crop of navel oranges in solid plantings.

The evidence from mixed plantings of the major varieties grown in California (Washington navel, Valencia, Eureka, Lisbon, Marsh) indicates that pollination by bees is probably a negligible factor in the production of these varieties. Wong (1939) reported no difference between cross- and self-pollination in Valencia oranges. Plaut (1947) got no increase in fruit set in Valencia with cross-pollination, although he did get an increase in seediness. Cameron, Cole, and Nauer (1960) got an apparent increase in fruit set in Valencia by cross-pollination. El-Tomi (1954, 1957*b*) reported improved yield in some years from cross-pollination of Washington navel.

In two varieties of satsuma, Nagai and Tanikawa (1928) obtained substantially the same set of fruit with no pollination, with self-pollination, and (one variety tested) with cross-pollination.

Although the presence of bees in solid or mixed plantings of some varieties may have no significant effect on yield, other varieties may benefit from the presence of bees even in solid plantings. In the absence of other pollen sources, Lacarelle and Miedzyrzecki (1937) and Van Horn and Todd (1954) reported increased fruit set when bees were placed in cages built over individual Clementine mandarin trees. Fujita (1957) got increased set with bee pollination in satsuma. Hassanein and Ibrahim (1959) got reduced set in Khalili orange when bees were excluded. Self-pollination by bees of flowers prior to normal anthesis may be a factor in this increased fruit set. Flower buds of some varieties often have the stigma exposed prior to anthesis. Iwasaki, Ohata, and Nishiura (1954) got increased seed set in the satsuma when flowers were pollinated at least four days prior to opening. Pereau-Leroy (1950) also got increased fruit set in Clementine by using bud pollination. Seedy fruit can also set by self-pollination of buds in at least some varieties of *C. grandis* that normally set only seedless fruit when self-pollinated (Soost, 1964).

With self-incompatible or pollen-sterile varieties possessing a low degree of parthenocarpy, the presence of other pollen sources and bees is essential to fruit production. Uphof (1934), who made comparative tests of self-pollination and cross-pollination on small branches of many varieties, found that fruit setting was commonly more abundant with crossing. He concluded that the abundant occurrence of defective pollen in some varieties seemed an adequate explanation of this result, and that some pollen produced too little growth of pollen tubes. This latter condition, if characteristic of selfing as compared with crossing, would indicate true self-incompatibility. Because of self-incompatibility (see pp. 313), cross-pollination is needed for adequate fruit production in Clementine mandarin, Orlando tangelo, Minneola tangelo, Robinson mandarin, Hyuganatsu, and several pummelo varieties. It is probably also needed in Sukega grapefruit and Osceola mandarin.

Pollination and Fruit Size

The number of seeds per fruit may affect fruit size, at least in some varieties. Krezdorn (1959) and Cameron *et al.* (1960) found significant correlations between fruit size and seed number in the Orlando tangelo and Valencia orange, respectively. Significant correlation or a marked trend toward correlation was also reported by Cameron *et al.* (1960) in a seedy sweet orange, Mediterranean Sweet orange, Trovita orange, and Frua mandarin. Minneola tangelo (Cameron *et al.*, 1960) and Clementine mandarin (Coste and Gagnard, 1956; Soost, 1956) also have higher seed numbers in the larger fruits. Because the number of seeds can be increased in several of these varieties by cross-pollination (see p. 313), adequate pollination can affect not only yield but fruit size. Hearn and Reece (1967) reported that Page mandarin fruits produced from self-pollination were seedless and too small for commercial use. Seedy fruits were produced by cross-pollinating with Lee mandarin pollen, producing larger fruits than either Orlando tangelo or Temple tangor pollen.

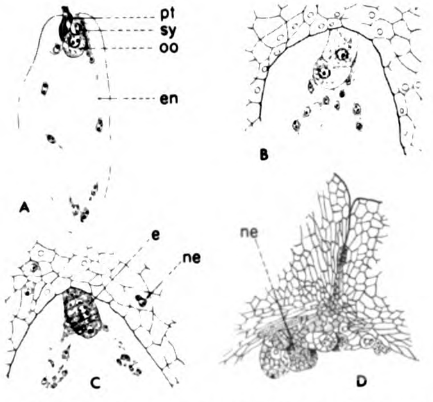

Fig. 4–8. Development of embryos and endosperm in *Poncirus* (*A-C*) and *Citrus sinensis* (*D*). *A*, embryo sac shortly after fecundation; *B*, micropylar end of embryo sac with two-celled zygotic embryo; *C*, six-celled zygotic embryo, development of nucellar embryos begun in nucellus; *D*, apex of older sac with many nucellar embryos, zygotic embryo not recognizable. Key to symbols: *e*, gametic embryo; *en*, endosperm [lining sac]; *ne*, nucellar embryos; *oo*, fecundated egg; *pt*, tip of pollen tube; *sy*, synergid. (*A-C*, redrawn from Osawa, 1912; *D*, redrawn from Strasburger, 1878.)

Pollen Tube

The thick, sticky liquid secreted by the stigma serves not only to catch and hold the pollen grains, but also provides suitable conditions for their germination. Climenko (1936), Singh and Dhuria (1960), Randhawa, Nath, and Choudhury (1961) reported that the stigma is receptive one to three days prior to anthesis and retains receptivity for six to eight days after anthesis. Bud-pollination (Fujita, 1957) has demonstrated successful pollen-tube growth as much as four days prior to anthesis. The pollen grain absorbs moisture from the stigmatic liquid and forms the pollen tube. Pollen tubes grow through the style to locules of the ovary. According to Webber (unpublished), the pollen tubes grow in the stylar canals. However, Banerji (1954) stated that growth is intercellular and not in the canals. In the ovary, a pollen tube grows through the micropyle of each ovule, disorganizing one or both synergids as it enters the embryo sac (Bacchi, 1943; Banerji, 1954).

Fecundation

When the pollen tube has reached the embryo sac by way of the micropyle, a sperm nucleus passes into the embryo sac, where it fuses with the egg nucleus, thus accomplishing fecundation. According to Banerji (1954), the second sperm nucleus fuses simultaneously with the two polar nuclei to produce a triploid endosperm nucleus. Toxopeus (1933) found 27-chromosome (triploid) endosperm in two diploid forms of *Citrus*. Figure 4–4, *E* and *F*, which show embryo sacs ready for fecundation, indicate that the polar nuclei themselves probably fuse (Osawa, 1912). Figure 4–8 shows the egg cell after fecundation has occurred and the endosperm nucleus has divided.

The period from pollination to fecundation has been reported to be from two days to four weeks. Strasburger (1878), Toxopeus (1930), and Osawa (1912) reported periods from two to four weeks. In Foster grapefruit, Bacchi (1943) found fecundation occurred in four days. Furusato (1953) reported a period of two to eight days for satsuma. Ikeda (1904–06) also observed that removal of the stigma two or three days after pollination did not prevent setting of seed. The shorter period to fecundation probably is more representative of *Citrus* when growing under favorable conditions. Pollen-tube growth could be altered greatly by environmental factors or by pollen incompatibility. Gerasimova-Navashina (1960) indicated that fusion of the gametes depends on temperature and the condition of the gametes.

DEVELOPMENT OF EMBRYO AND ENDOSPERM

The Gametic Embryo

Strasburger (1878) stated that in greenhouses in Germany the egg, fecundated in the fall, usually overwintered before dividing, but that in southern countries development was uninterrupted, Schacht finding that on the Madeira Islands off the coast of Morocco a mandarin variety showed the first division about two months after flowering. Osawa (1912) found that in *Poncirus* the first division of the egg took place between two and four weeks after fecundation (fig. 4–8). Bacchi (1943) stated that the first division occurs about fifty days after fecundation, and Furusato (1953) indicated a period of about one month. The first divisions are transverse (fig. 4–8); the cells nearest the micropylar end of the embryo sac form the slender suspensor, and the apical cell at the free end of the row divides to form a rounded mass of cells. Later outgrowths initiate the various parts of the complete embryo: hypocotyl, radicle, cotyledons, and plumule.

Nucellar Embryos

In many varieties of *Citrus,* and also in *Fortunella* and *Poncirus,* extra embryos, derived not from cells of the embryo sac but from somatic cells of the nucellus, are developed in the ovules. These grow into the embryo sac and lie alongside the normal embryo. After fecundation, and before or soon after the egg undergoes its first division (Strasburger, 1878; Biermann, 1896; Osawa, 1912; Toxopeus, 1930; Bacchi, 1943; Furusato, 1953; Vasiljcova, 1951), stained sections of ovules show a few large cells, with large nuclei and dense cytoplasm, in the nucellus. These cells are mainly near the micropylar end of the embryo sac and within one or two cell layers of it (fig. 4–8, *C*). Some of these cells begin to divide and produce small masses of cells which protrude into the sac alongside the embryo derived from the egg cell (fig. 4–8, *D*). While very young, these embryos may be distinguishable by their irregular shape and lack of a suspensor. However, Maheshwari and Ranga-Swamy (1958) reported the presence of suspensors. These extra embryos are called nucellar embryos (Toxopeus, 1930). The time relationship between the initiation of development of the zygotic embryo and the nucellar embryos may be quite variable. Biermann (1896) concluded that at an early state the nucellar embryos are less advanced than the zygotic embryo. Toxopeus (1930) and Vasiljcova (1951) indicated that most of the nucellar embryos were several-celled when the egg began to divide.

Endosperm

Before the egg cell divides, the embryo sac begins to enlarge rapidly, crowding and destroying many cells of the nucellus. The endosperm nucleus divides, and successive divisions form many free nuclei, which become scattered through a thin layer of cytoplasm that lines the embryo sac (fig. 4–8). Bacchi (1943) found the free nuclear condition still present 67 days after pollination. Cell walls then form, and these cells divide further to form the endosperm. In *Citrus* and closely related genera, the endosperm transports nutrients for the developing embryos.

Embryo Culture

Embryo culture has been used as an aid in breeding and in investigating various aspects of nucellar embryony. Sobrinho and Gurgel (1953) reported growth of 2 mm embryos in "Sachs" solution at pH 5. Stevenson (1956) reported shoot and root growth of embryos extracted from fruits within five to six months after pollination and placed on nutrient agar. Hurosvili (1957) got satisfactory growth on Tukey's medium at pH 7 to 7.5 with vitamins B_1 and B_2 added. Ohta and Furusato (1957*a*) unsuccessfully attempted to culture small embryos (less than 1 mm) from mature seeds. Embryos larger than 3 mm grew best on nutrient agar without sucrose at pH 6. Addition of sucrose caused growth reduction. They further reported that addition of 0.01 ppm of vitamin C or vitamin B produced increased growth, but higher concentrations retarded growth. The addition of 2,4,5-T may have increased growth at 1 ppm or less; however, no stimulation of 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthaleneacetic acid (NAA) was noted and concentrations of NAA greater than 0.1 ppm retarded growth. Ozsan and Cameron (1963) found 2 per cent sucrose better than 1 per cent for growth of 1- to 3-mm embryos, but 1 per cent was better than 2 per cent for 4- and 5-mm embryos. Absence of sucrose reduced growth of all embryos. With 2- and 4-mm embryos, root growth was much greater when only sucrose was added to the basic nutrient agar. For 1-mm embryos, growth was best when immature coconut milk and a combination of growth factors were added in addition to sucrose. The inorganic media of Tukey and Nitsch were better than those of Randolph and Cox or White (modified). A 0.7 per cent agar was used with all media. Ranga-Swamy (1959) reports enlargement and differentiation of "proembryos" on 5 per cent sucrose, but not on 2 or 3 per cent when they were included with extracted ovules. Proembryos extracted from ovules did not grow on White's medium. Sabharwal (1962) was unable to induce the development of nucellar embryos *in vitro* unless they had been initiated prior to the extraction of the nucelli.

Dr. Toshio Murashige and co-workers of the Citrus Research Center and Agricultural Experiment Station, Riverside, California, are currently developing culture techniques that may provide a method of obtaining nucellar embryos from the nucellus of varieties that normally do not produce nucellar embryos. Nucellar tissue extracted after the initiation of the zygote has differentiated on a defined nutrient medium. Partially developed zygotes extracted from varieties that normally produce very few sexual seedlings have also differentiated on the same medium.

POLYEMBRYONY

Polyembryony is the development of two or more embryos in one seed. In *Citrus,* extra embryos are produced in two ways: (1) commonly from cells of the seed parent (nucellar embryony); and (2) occasionally by the production of two or more zygotic embryos, either from fission of one fecundated egg or from two or more functional embryo sacs in a single ovule (Bacchi, 1943).

Multiple Zygotic Embryos

In several cases among hybrids produced at Riverside, California, two separate seedlings grew from one seed. In each case, the two hybrids seem identical in genetic type, although such identity has not been observed elsewhere among citrus hybrids. It is most likely that each of these pairs of "twins" were produced by the development of two embryos from one fecundated egg. Twin hybrids were also found in Swingle's seedling populations (Traub and Robinson, 1937).

Traub and Robinson (1937) also reported one triple hybrid and one quadruple hybrid in Swingle's populations. Ozsan and Cameron (1963) proved in four cases that three seedlings from the same seed were hybrid. These multiple embryos could be produced by multiple fission of the fecundated egg or by the functioning of more than one embryo sac per ovule (see also chap. 5).

Nucellar Embryony

Since nucellar embryos develop asexually by ordinary mitotic division of cells of the nucellus, no male cells contribute to their formation, and no reduction division occurs in the seed-parent cells which give rise to them. Nucellar seedlings therefore not only inherit from the seed parent alone, but are actually identical with it in genetic constitution, except for possible differences due to somatic (bud) variation. This asexual reproduction has very important consequences for evolution, breeding, and culture of the citrus fruits, all of which is discussed in Chapter 5.

Terminology of Nucellar Embryony.—The term nucellar embryony (Ernst, 1918; Sharp, 1934) is especially suitable because it precisely indicates what occurs in *Citrus* and its near relatives, and includes nothing else. Embryos which develop from the nucellus may appropriately be called nucellar embryos, and trees which grow from these embryos are nucellar seedlings; these embryos and seedlings are the nucellar progeny or offspring of their parent.

The more inclusive terms apomixis and apomictic are obviously applicable to nucellar embryony. They are preferable to apogamy and apogamic, which have sometimes been employed for *Citrus,* but which are commonly restricted to formation of embryos from gametophytic cells.

Other terms for the nucellar embryos are: false, extra, adventive, adventitious, asexual, and vegetative. Terms applied to embryos developed from the egg are: true, normal, sexual, generative, zygotic, and gametic; of these, zygotic seems to be the most satisfactory, since it precisely indicates their origin, except in possible instances of parthenogenesis.

Relation of the Pollen to Nucellar Embryony.—The available evidence on the initiation of the development of nucellar embryos indicates that pollination is usually, but perhaps not invariably necessary. The usual seedlessness of the ordinary varieties of navel oranges, satsuma mandarin and Tahiti lime in solid plantings indicates that in these varieties seeds do not often form, if ever, without pollination by varieties having good pollen. Such varieties show that auxin may be adequate for fruit setting without inducing nucellar embryony. Furusato and Suzuki (1955) produced only seedless fruits when using 2,4,5-T. Only seedless fruits were obtained by Soost (1958) by application of gibberellin to unpollinated Clementine fruit.

Except for two reports (Webber, 1930; Wright, 1937), seed formation has not been observed when pollen has been excluded. Ikeda (1904–06) and Coit (1915) found that prevention of pollination resulted in seedlessness when any fruits were produced. Nagai and Tanikawa (1928) emasculated and bagged, without pollination, flowers of eighteen citrus varieties which produced, when self-pollinated, averages ranging from one to forty-three seeds per fruit. Of these eighteen varieties, thirteen produced some fruits without pollination, but all these fruits were seedless. Wong (1939) found that four varieties, which commonly have seeds if pollinated, produced only seedless fruits when pollen was excluded. Toxopeus (1930) obtained no fruits when pollination was prevented in several hundred flowers of three seedy varieties.

With certain interspecific hybrids, which rarely, if ever, produce zygotic seedlings, pollination seems to be necessary for the formation of nucellar embryos (Swingle, 1927). The fact that the seed production of some such hybrids is much greater when suitably cross-pollinated than when they are self-pollinated also indicates dependence of nucellar embryony on pollination with functional pollen; the Thomasville citrangequat and the Morton citrange are examples.

Although there may be exceptions, pollination is unquestionably usually necessary for the formation of nucellar embryos.

Whether or not fecundation is a prerequisite for the formation of nucellar embryos is not clear. Several varieties which seem to be self-sterile (p. 313) occasionally produce fruits having "imperfect seeds with poorly developed embryos" after self-pollination or evidently incompatible cross-pollination (Nagai and Tanikawa, 1928). The embryos in these seeds may be nucellar embryos which result from pollen-tube stimulus, without fecundation and therefore without normal endosperm development. Toxopeus (1930) obtained no fruits when pollen of *Feronia,* a near relative of *Citrus,* was used on *Citrus*. This lack of fruit set could be due to lack of pollen-tube growth rather than lack of fecundation. The initiation of nucellar embryos after fecundation has indicated the need for fecundation in their development. However, it is not clear that some development does not occur without fecundation.

Johri and Ahuja (1956) described the development of endosperm and nucellar embryos in embryo sacs of *Aegle marmelos* (L.) Corr., a *Citrus* relative, in which the egg and antipodal cells degenerate. Presumably, union of polar nuclei and a sperm cell occurred. If such a phenomenon occurs in *Citrus,* nucellar embryos could develop without the initiation of a sexual embryo.

Interrelations of the Two Classes of Embryos

Except in the forms which rarely or never produce nucellar embryos, the zygotic embryo must often or usually compete for space and nutrients with one or more nucellar embryos. Strasburger (1878), Bacchi (1943), and Furusato (1953) observed that embryo sacs may contain one, two, or sometimes more embryos developing normally, together with others that are more or less sup-

Table 4–1

COMPARISON OF MEAN NUMBERS OF EMBRYOS AND SEEDLINGS

Variety or Species	Embryos Per Seed	Seedlings Per Seed	Seedlings Per Embryo
Kishiu mandarin	1.02	1.00	0.98
King mandarin	1.10	1.03	0.94
Triumph grapefruit	1.18	1.08	0.92
Ellen grapefruit	1.32	1.20	0.91
Citrus aurantium[*]	1.50	1.03	0.69
Tison mandarin	1.64	1.08	0.76
McCarty grapefruit	1.70	1.23	0.72
Rangpur lime[*]	1.70	1.08	0.63
Szinkom mandarin	1.78	1.38	0.76
Batangas mandarin	2.01	1.23	0.61
Ladu mandarin	2.64	1.78	0.67
China mandarin	2.72	1.42	0.52
Citrus sinensis[*]	2.79	1.31	0.47
Rough lemon	2.90	2.00	0.69
Calamondin[*]	3.59	1.70	0.47

[*] Data from Moreira, Gurgel, and de Arruda (1947); all other entries from Torres (1936). Other data presented by the authors indicates much variation within the varieties.

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Table 4–2

NUMBERS OF ZYGOTIC AND NUCELLAR SEEDLINGS FROM VARIOUS SEED-PARENT SPECIES AND VARIETIES, WITH CROSS-POLLINATION

Seed Parent	Source of Data*	Number of Pollen-Parent Varieties†	Number of Seeds	All Seedlings		Zygotic‡ Seedlings		Nucellar Seedlings	
				Total	Per Seed	Total	Per Seed	Total	Percentage of All Seedlings
Lemon:									
Eureka	CRC	5	210	223	1.06	150	0.71	73	33
Lisbon	CRC	6	703	741	1.05	500	0.71	241	32
Villafranca	Torres	5	132	143	1.08	66	0.50	77	54
Italian	Toxopeus	3	255	306	1.20	168	0.66	138	45
Feminello	Russo and Torrisi	2	23	33	1.43	8	....	18	69§
Monachello	Russo and Torrisi	2	281	306	1.09	155	....	49	24§
Rough	Torres	1	819	1,607	1.96	38	0.05	1,569	98
Rough	CRC	1‖	127	158	1.24	72	0.57	86	54
Lime:									
Mexican	CRC	1‖	14	18	1.29	4	0.29	14	78
Kusaie	CRC	1‖	87	95	1.09	70	0.80	25	26
Red	CRC	1‖	130	152	1.17	86	0.66	66	43
Mandarin group:									
Dancy	CRC	6	193	265	1.37	0	0.00	265	100
China#	Torres	3	434	868	2.00	151	0.35	717	83
Willowleaf	CRC	7	771	984	1.28	138	0.18	846	86
Avana#	Russo and Torrisi	2	105	168	1.60	8	....	62	89§
Kishiu	Torres	6	333	333	1.00	333	1.00	0	0
Frua	CRC	1	73	90	1.23	4	0.05	86	95
Ponkan	CRC	1	79	113	1.42	2	0.02	111	98
Kara	CRC	1	83	142	1.71	0	0.00	142	100
Szinkom	Torres	7	1,683	2,521	1.50	421	0.25	2,100	83
King	CRC	7	387	391	1.01	308	0.80	83	21
King	Torres	8	2,006	2,085	1.04	2,006	1.00	79**	4
Satsuma (Owari?)	CRC	11	323	464	1.44	46	0.14	418	90
Grapefruit:									
Marsh††	CRC	6	207	223	1.08	10	0.05	213	96
Imperial‡‡	CRC	8	626	788	1.26	264	0.42	524	66
Imperial	CRC	Selfing	99	121	1.22	10	0.10	111	92
Panuban	Torres	1	70	78	1.11	58	0.83	20	26
Sukega§§	CRC	2	116	116	1.00	116	1.00	0	0

Pummelo:									
Four varieties	Torres	14	1,954	1,955	1.001	1,955	1.001	0	0
Seven varieties	CRC	8	610	610	1.00	610	1.00	0	0
Sweet orange:									
Maltese Oval	CRC	8	167	182	1.09	111	0.66	71	39
Paperrind	CRC	5	82	128	1.56	21	0.26	107	84
Ruby	CRC	6	183	252	1.38	61	0.33	191	76
Valencia	CRC	8	106	143	1.35	22	0.21	121	85
Ruvel	CRC	5	65	86	1.32	7	0.11	79	92
Washington navel	CRC	6	24	32	1.33	1	0.04	31	97
Washington navel	Torres	1	32	64	2.00	20	0.62	44	69
Sour orange:									
Sour orange	Torres	1	319	385	1.21	56	0.18	329	85
Tangelo:									
Orlando	CRC	1	525	690	1.31	105	0.20	575	83
Minneola	CRC	1	279	416	1.49	11	0.04	405	97
Pearl	CRC	1	75	105	1.40	7	0.09	98	93
Sunshine	CRC	1	401	698	1.74	0	0.00	698	100
C. taiwanica Tan. & Shimada	CRC	1[‖]	594	755	1.27	403	0.68	352	47
C. macrophylla Hook.	CRC	1[‖]	139	198	1.42	2	0.01	196	99
C. amblicarpa Ochse	CRC	1[‖]	54	69	1.28	0	0.00	69	100
C. ichangensis Swing.	CRC	1[‖]	11	11	1.00	11	1.00	0	0
C. pennivisiculata Tan.	CRC	1	110	150	1.36	6	0.05	144	96
Yuzu	CRC	1[‖]	90	112	1.24	37	0.41	75	67
Yuzu	CRC	1[‖]	198	242	1.22	11	0.05	231	95
Ichang hybrid	CRC	1[‖]	231	366	1.58	0	0.00	366	100
P. trifoliata (L.) Raf.	CRC	1	228	236	1.03	203	0.89	32	13
P. trifoliata	CRC	Selfing	80	101	1.26	8	0.10	74	73

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[*] Data used are from records of the Citrus Research Center, Riverside (CRC), Torres (1938), Russo and Torrisi (1953), and Toxopeus (1930).

[†] Except where selfing is indicated.

[‡] The classification of zygotic seedlings for CRS data is based on fruiting trees or on progenies with *P. trifoliata* as the pollen parent. The classifications by Toxopeus (1930), Torres (1938), and Russo and Torrisi (1953) were apparently on young seedlings.

[§] Based on number of seedlings surviving at the time of classification.

[‖] *P. trifoliata* used as the pollen parent. Zygotic seedlings identified by presence of trifoliate leaves.

[#] Presumably the same as Willowleaf.

[**] Torres recognized no nucellar seedlings from King seeds producing only one seedling each. At Riverside, such seeds gave about 20 per cent of nucellar seedlings.

[††] Includes data from strain listed as "Seedy Marsh."

[‡‡] A variety unlike standard commercial varieties.

[§§] A hybrid of uncertain parentage.

pressed. In table 4–1 data are given illustrating the elimination of embryos at germination. The result of this competition must depend on the number of nucellar embryos, their time of starting, their location, and the relative inherent or genetic vigor of the two kinds of embryos. Reports by Sokol'skaja (1938) and Majsuradze (1961) indicated that the lower the number of embryos per seed, the larger the average size of the embryos and the greater the probability of hybrids surviving. In many varieties, a seed produces only one or more nucellar seedlings, without a gametic seedling, indicating that the zygotic embryo (if ever formed) was crowded out or was simply too weak to survive. When the zygotic embryos fail to survive in all or most of the seeds, the seedlings produced will be all or nearly all from nucellar embryos, and the seed parent will "breed true" with respect to all or nearly all of its offspring (Frost, 1926; Toxopeus, 1936).

Toxopeus (1936) mentioned three factors as being relatively unfavorable to the zygotic embryos: (1) in the material which he examined, the fecundated egg cell began to divide when most of the nucellar embryos already consisted of several cells each; (2) the young zygotic embryo, situated near the apex of the embryo sac, seems less favorably placed than the nucellar embryos, with respect both to transport of nutrients from the vascular system and to the opportunity to occupy the space within the sac; and (3) zygotic seedlings for the most part are weaker in genetic constitution than the corresponding nucellar seedlings, and the zygotic embryos which survive to produce seedlings are probably more vigorous, on the average, than those which are crowded out. Evidence indicates that the pollen parent may influence the relative proportions of zygotic and nucellar progeny (Toxopeus, 1931, 1936; Torres, 1936; Frost, 1926; Mamporija, 1957; Lomija, 1961*a*, 1961*b*; Soost and Cameron, unpublished). Toxopeus (1936) concluded that this is most likely a result of unlike influences of different kinds of pollen on the development of nucellar embryos. However, differences in the vigor of the hybrids produced may be the main cause. Environmental and internal factors undoubtedly also affect the proportions of zygotic and nucellar seedlings. In breeding work at Riverside, California, with the Orlando tangelo, using a single variety of pollen, a few fruits produced over 10 per cent zygotic progeny while all other fruits produced only nucellar progeny.

The proportions of zygotic and nucellar progeny resulting from cross-pollination of various varieties (table 4–2) have been reported by Webber (1900), Frost (1926), Toxopeus (1930), Torres (1932, 1936, 1938), Brieger and Moreira (1945), and Russo and Torrisi (1953). The proportions from self or open pollination have been discussed by Frost (1926), Toxopeus (1930), Webber (1932), Torres (1936), Moreira and Gurgel (1941), Naik (1949), and Russo and Torrisi (1953). Additional unpublished information on the proportions of zygotic and nucellar progeny has been obtained in breeding programs of the Citrus Research Center at Riverside, California, and of the U.S. Department of Agriculture at Orlando, Florida, and Indio, California.

Numbers of Embryos and Seedlings

The number of embryos in the mature seeds has been reported by Frost (1926), Toxopeus (1930), Torres (1936), Navarro de Andrade (1933), Brieger and Moreira (1945), Nasharty (1945), Moreira, Gurgel and de Arruda (1947), Gurgel and Sobrinho (1951), Py (1951), Minessy (1953), Furusato (1953), Russo and Torrisi (1953), Chapot and Praloran (1955), Furusato, Ohta and Ishibashi (1955), Furusato (1957), Minnessy and Higazy (1957), Parlevliet and Cameron (1959), and Ozsan and Cameron (1963). Additional information on the number of seedlings per seed has been obtained in breeding programs at the Citrus Research Center, Riverside, California, and from the U.S. Department of Agriculture at Orlando, Florida, and Indio, California. Tanaka (1954) indicates whether or not many of the forms he discusses are polyembryonic.

The number of embryos per seed varies greatly even on one tree, the average number differs greatly according to variety, and there is no general consistency within many of the species in which polyembryony is present. Nasharty (1945), Chapot and Praloran (1955), and Minessy and Higazy (1957) presented data showing rather large differences observed between localities in the same year. Moreira *et al.* (1947) and Minessy and Higazy (1957) demonstrated differences between years. Moreira *et al.* (1947) also analyzed the variability between trees, fruits, and seeds. Nasharty (1945) and Minessy (1953) also indicated an effect of rootstock with some scions. Undoubtedly, many environmental and other factors affect the number of embryos per seed. Traub (1936) reported a decrease in the average number of embryos by decreasing the food supply. Furusato (1953) reported a reduction in the number of embryos per seed after injection of young fruit with either water or maleic hydrazide-30. Furusato *et al.* (1955) reported significantly higher numbers of embryos per seed on the north side of trees and in old (thirty-year-old) trees.

The embryos in a polyembryonic seed often differ widely in size and shape (fig. 4–9). The average number of seedlings produced per seed is commonly much smaller than the total number of embryos, and the seedlings are doubtless derived mainly from those embryos that are comparatively large (table 4–3). Although some varieties have more than two embryos in the great majority of their seeds, in most varieties few seeds produce more than two or three seedlings each. Table 4–2 presents the percentages of nucellar seedlings for representative varieties. Because of considerable confusion in the nomenclature of citrus varieties and species, the degree of polyembryony of any accession should be checked unless its identity is well known.

In *C. limon,* the Eureka and Lisbon lemons have only one embryo in most of the seeds and give low percentages of nucellar seedlings (Frost, 1926). Russo and Torrisi (1953) reported low percentages of nucellar seedlings for Monachello, but rather high percentages for Feminello.

Rough lemon, classified as a separate species by Tanaka (1954), has a relatively large number of embryos, and usually has a high percentage of nucellar seedlings. When *P. trifoliata* pollen was used, 40 per cent gametic seedlings were obtained (Soost, unpublished). Meyer lemon is monoembryonic, and no nucellar seedlings have been reported. Ponderosa lemon and sweet lemon (Moreira *et al.,* 1947) have a very low degree of polyembryony.

Table 4–3

MEAN NUMBERS OF EMBRYOS AND SEEDLINGS PER SEED PRODUCED BY SIX CITRUS VARIETIES IN JAVA

Variety[*]	All Embryos	Large Embryos	Seedlings
Japanese lemon (*C. hybrida*)	1.64	1.14	1.20
Italian lemon (*C. medica*)	2.15	1.12	1.20
Djeroek nipis (*C. aurantifolia*)	2.07	1.06	1.07
Djeroek eter (*C. nobilis*)	6.10	1.94	1.45
Djeroek kastoeri (*C. mitis*)	8.96	1.60	1.62
Rough lemon (*C. hybrida*)	4.58	1.45	1.70

Source: Toxopeus (1930).

[*] English translations are given for Dutch names; Javanese and taxonomic names are as given by Toxopeus. *C. hybrida* was used to designate presumed hybrids. Japanese lemon is the equivalent of Rangpur lime. *C. medica* L. as used here is equivalent to *C. limon* (L.) Burm. f.

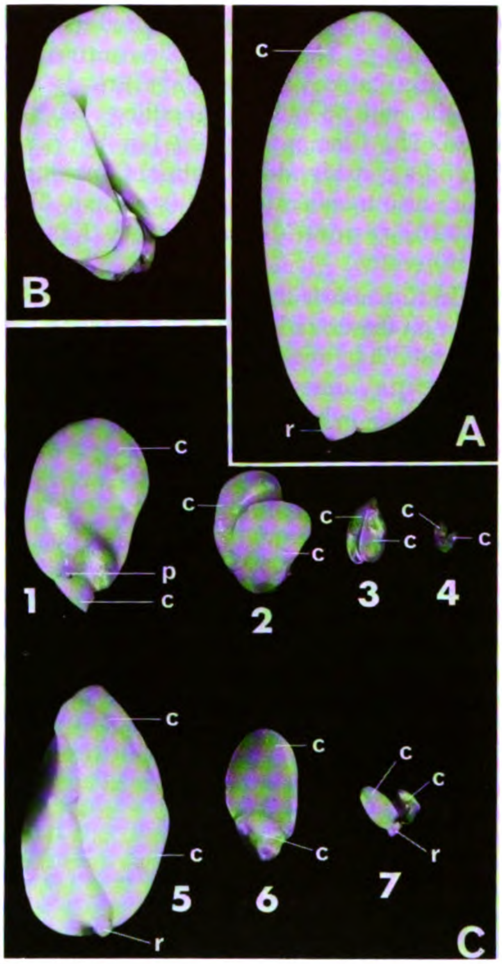

Fig. 4–9. ***A,*** **single embryo from monoembryonic *C. grandis; B,* multiple embryos from polyembryonic *C. reticulata* Blanco cv. Ponkan; *C,* seven embryos, each with two cotyledons, from one seed of Ponkan. Key to symbols: *c,* cotyledons; *p,* plumule; *r,* radicle.**

In *C. reticulata,* many varieties, including Dancy, Ponkan, Willowleaf, and satsuma, have numerous embryos and high proportions of nucellar seedlings. The King has few extra embryos and usually gives low proportions of nucellar seedlings from cross-pollination, but the Japanese variety Kunenbo, very similar to King, is usually polyembryonic (Tanaka, 1954). Kinnow and Kara, which have King as one parent, have many embryos per seed and produce few, if any, gametic seedlings. However, Wilking and Kincy, also hybrids having King as one parent, are monoembryonic and have not been known to produce any nucellar seedlings. Temple and Clementine, which are hybrids of unknown parentage, are also monoembryonic and no nucellar seedlings have been reported. The Clement tangelo, Lee hybrid mandarin, and a considerable number of unnamed hybrids are also monoembryonic (see chap. 5). Torres (1936) reported the variety Kishiu as monoembryonic.

In *C. sinensis* the number of embryos may be either moderate or high, and the proportions of nucellar seedlings are rather high in most varieties. No monoembryonic varieties have been reported.

Various grapefruit varieties *(C. paradisi)* produce many nucellar embryos. Several ordinary varieties have given high proportions of nucellar seedlings, whereas the Imperial, which is decidedly unlike the usual commercial varieties, has given a medium proportion. Torres (1932) found a low proportion in the variety Panuban. Sukega, a hybrid grapefruit, is monoembryonic (Parlevliet and Cameron, 1959), and no nucellar seedlings have been identified. The Wheeny grapefruit, which appears to be a hybrid, is also monoembryonic. Moreira *et al.* (1947) report some questionable grapefruit as monoembryonic.

The pummelos *(C. grandis)* have been reported to be monoembryonic by most investigators (Toxopeus, 1930, 1933; Torres, 1936; Brieger and Moreira, 1945; Nasharty, 1945; Py, 1951). No nucellar seedlings have been produced at the University of California Citrus Research Center, Riverside, using *C. grandis* as a seed parent in the production of several hundred hybrids. Frost found a high percentage of nucellar embryos in the Frizzelle pummelo, and other *C. grandis* accessions at Riverside have also been polyembryonic. Because *C. grandis* hybridizes readily with other citrus and many of the hybrids are pummelo-like in appearance, these polyembryonic varieties could well be hybrids.

Although *C. aurantium* may give rather low numbers of embryos, the proportion of nucellar seedlings is high.

The citrons (*C. medica* L.) appear to be mainly monoembryonic (Moreira *et al.*, 1947; Py, 1951; Tanaka, 1954; Chapot and Praloran, 1955; and Carpenter, unpublished).

The many diverse types classified as limes (*C. aurantifolia* Christm.) Swing., including the so-called mandarin-limes (*C. limonia* Osbeck) generally have fairly low numbers of embryos per seed but usually produce low proportions of hybrid seedlings.

Although Tanaka (1954) indicated a number of his reported species were monoembryonic, it is not always clear whether they are strictly monoembryonic and produce no nucellar embryos. His *C. junos* Sieb. ex Tan. is indicated to be monoembryonic, yet correctly identified accessions of this species at Riverside produce nucellar seedlings.

Torres (1930, 1936), Nasharty (1945), Moreira *et al.* (1947); Chapot and Praloran (1955), and Parlevliet and Cameron (1959) found occasional extra embryos or seedlings in varieties usually producing only one embryo per seed and not known to have produced nucellar seedlings. Ozsan and Cameron (1963) obtained seed in three of these monoembryonic varieties (Clementine, Wilking, and Siamese pummelo) by guarded cross-pollination with *P. trifoliata,* which imparts a dominant trifoliate leaf to its hybrids. All nucellar seedlings would therefore be monofoliate. Of a total of 131 embryos, excised from 50 seeds with more than one embryo each, 69 were determined to be zygotic. The remaining 62 embryos did not grow large enough to be judged. No seedling was shown to be nucellar. In 14 cases, two embryos from the same seed were shown to be zygotic; in four cases, three from the same seed were proved so. The data strongly indicates that these three varieties do not produce nucellar embryos, and suggest that other known cases of extra embryos in varieties normally producing only zygotic progeny are also not of nucellar origin.

THE SEED

During the later stages of embryonic development the endosperm and the nucellus almost disappear, leaving only vestiges which contribute to the formation of the inner seed coat.

Embryos

The cotyledons, in which most of the food supply for the germinating seedlings is stored, are fleshy and constitute by far the largest part of the mature embryo (fig. 4–9). They are attached to a very short hypocotyl, at the other end of which is the rudimentary radicle. Lying between the cotyledons is the plumule, which until germination begins is merely a minute cone that has not yet developed the first true leaves (Casella, 1931). Martin (1946) classified the embryo as investing.

The one or more embryos form a more or less solid, rounded-to-corrugated mass, in which the radicles normally point toward the micropylar end of the seed. A seed with one embryo usually has two cotyledons about equal in size and shape, but when there are two or more embryos the size and shape of their cotyledons often vary greatly (fig. 4–9). Occasionally an embryo has three or more cotyledons. When a seed contains several embryos, usually part of these are poorly developed, often with excessively small cotyledons (fig. 4–9). (See also Chapot and Praloran, 1955.) In polyembryonic varieties, the embryos are commonly crowded together at the micropylar end, but sometimes are located away from it (Nasharty, 1945; Parlevliet and Cameron, 1959). Sometimes a small embryo lies in a hollow in the outer side of a large cotyledon, or between two large cotyledons.

The color of the cotyledons may be creamy white, cream, or various shades of green. Intense green color is characteristic of the tangerines and mandarins, but some varieties such as the King only show occasional green. Chapot and Praloran (1955) described the satsuma as varying from deep pistachio green to white. They reported that oranges *(C. sinensis)*, sour orange *(C. aurantium)*, grapefruit *(C. paradisi)*, pummelos *(C. grandis)*, limes *(C. aurantifolia)*, and citrons *(C. medica)* have white cotyledons. Varieties that are normally white may develop a greenish color in overripe fruit. Chapot and Praloran (1955) particularly noted this behavior in *C. limon.*

Seed Coats

The tegmen, or inner seed coat, is a thin, rather delicate membrane which closely invests the embryo or embryos. The inner integument of the ovule probably forms the main part of the tegmen. The tegmen must include whatever remains of the nucellus and the endosperm. In most varieties, the tegmen is characteristically colored. The area covering the chalazal end is usually purple, brown (various shades), pink, yellow, or cream and the rest of the tegmen is usually lighter shades of these colors or grayish white (Chapot and Praloran, 1955). Chapot and Praloran suggested the use of the tegmen color, particularly at the chalazal end, as an identifying characteristic for the major groups of cultivated varieties and for relating uncertain varieties to these groups. Chapot and Praloran (1955) reported the association of the color of the chalazal end of the tegmen with the coloring of other plant parts and the acidity of the fruits. *C. limon* and acid varieties of *C. medica* are reported to have purple color in the flowers, new shoot growth, and chalazal end of the tegmen. In contrast, the non-acid citrons *(C. medica)* have white flowers and no purple color in the new shoot growth or in the tegman.

The outer seed coat or testa is usually grayish white, cream, or yellow in color and tough and woody in texture (figs. 4–10 and 4–11). In *Citrus,* the testa is often considerably wrinkled or ridged and is usually extended beyond the rest of the seed at one or both ends (especially the micropylar end) to form a conical beak or a flat plate which may extend along the whole length of the seed. In some polyembryonic seeds, the surface may be corrugated because of the unequal development of the embryos. In *Poncirus trifoliata,* Moreira *et al.* (1947) correlated the degree of

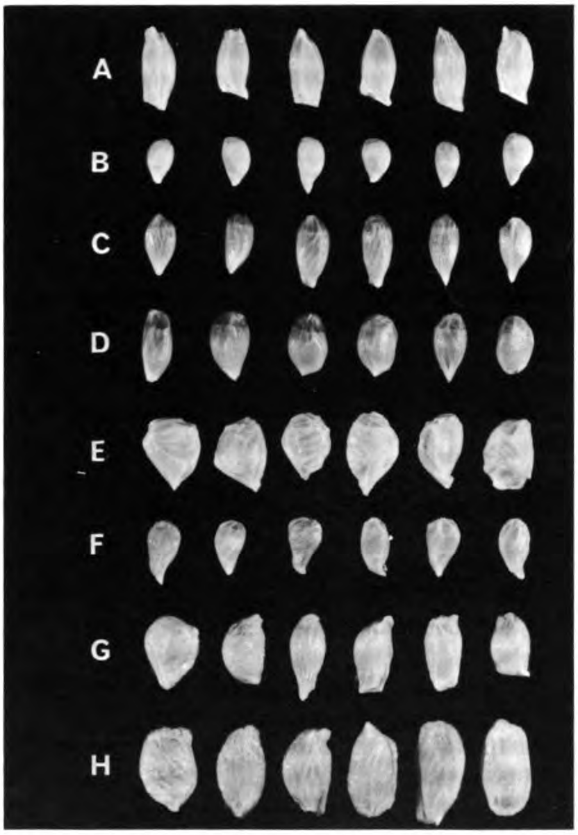

Fig. 4-10. Seeds of *Citrus* species. *A, C. aurantium,* standard sour orange; *B, C. aurantifolia,* Mexican lime; *C, C. limon,* Lisbon lemon; *D, C. limon,* Tetraploid Lisbon lemon; *E, C. paradisi,* Marsh grapefruit; *F, C. reticulata,* Dancy tangerine; *G, C. sinensis,* Koethen; *H,* hybrid grapefruit, Sukega.

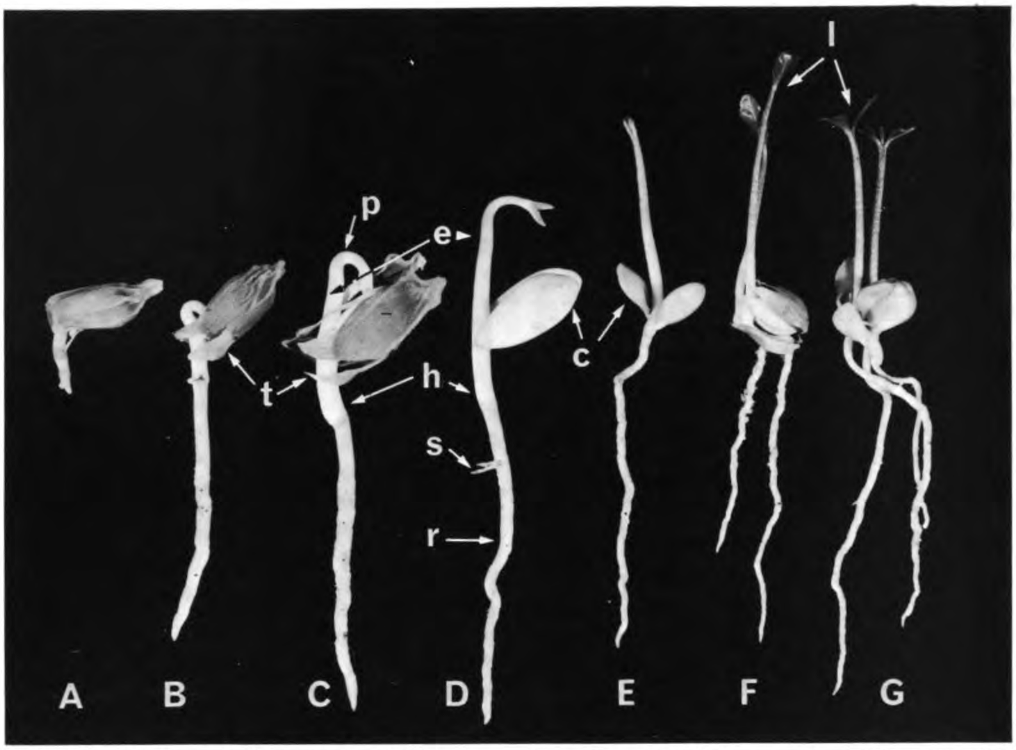

Fig. 4–11. Germination of citrus seeds. *A-D*, monoembryonic seeds of *C. grandis; E-G*, seeds of *C. reticulata*, showing polyembryony. Key to symbols: *c*, cotyledons; *e*, epicotyl; *h*, hypocotyl; *l*, first leaves; *p*, plumules; *r*, primary root; *s*, secondary roots; *t*, testa.

corrugation with the number of embryos. The surface of the testa is mucilagenous, so that the fresh seeds are very slippery. In *P. trifoliata*, the testa is coriacious and much less mucilagenous than in *Citrus* (Chapot and Praloran, 1955).

Seed Color

The color of the intact seed depends chiefly on the color of the tegmen and the embryos and the extent to which their color is visible through the testa; in a variety which has green cotyledons, the green color may be distinctly visible through the seed coats. Either drying or soaking in water changes the color of seeds by changing the opacity of the testa. Fresh seeds, in general, range from grayish white, cream, or yellow to brownish or greenish, with characteristic differences manifest between varieties (Casella, 1931; Chapot and Praloran, 1955).

Size and Shape of Seeds

Seeds of a given variety may be highly variable in size (Casella, 1931; Chapot and Praloran, 1955). The size and shape of the seeds is also affected by the number of seeds per fruit. Chapot and Praloran (1955) described seed shape as lenticular, cuniform, ovid, fusiform, almond, spherical, and modifications of these shapes (fig. 4–10). They indicate typical shapes for each of the species containing cultivated varieties. Some varieties also have characteristic shapes which aid in their identification. Chapot and Praloran (1955) suggest the use of seed shape to determine parentage of unknown hybrids.

Germination

The cotyledons are normally hypogean, remaining in the soil during germination. They absorb moisture and swell, and the tip of the radicle elongates and emerges through a break in the micropylar end of the testa (Casella, 1931). The radicle elongates rapidly to form a thick, fleshy taproot, which grows directly downward (fig. 4–11).

Meanwhile the plumule, so rudimentary in the resting seed, has been rapidly lengthening its first internode (the epicotyl) and developing the first two true leaves. The cotyledons lengthen

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somewhat, and after the taproot has made considerable growth the plumule emerges from the burst testa (fig. 4–11) and grows upward. Its tip is bent while pushing through the soil but as soon as it emerges it straightens up (fig. 4–11).

The first true leaves rapidly enlarge and soon separate. Usually there are two of these leaves; they are situated opposite on the stem and are somewhat unlike the later leaves in shape, so that they may be mistaken for cotyledons. Even in forms with normally trifoliate leaves, the first leaves are often unifoliate. Except in albinistic plants (see the chapter on propagation in vol. III), which remain entirely or partly whitish, the first leaves are yellow in the soil and become green very rapidly after they emerge. They soon reach their full size, and at that time germination may be considered complete.

Secondary roots do not appear until the taproot is some three or four inches (8 or 10 cm) in length and the first leaves are considerably developed (Casella, 1931).

Both root and stem normally emerge promptly at the micropylar end of the seed, but very often the tough testa obstructs the tip of the root so that it becomes bent or twisted before emerging. The position of the embryo in relation to gravity may also produce bent roots (Nasharty, 1945).

Many environmental factors, diseases, and pests affect the seeds during storage and after planting. For a discussion of the relations of these factors to seed handling, see Chapter 1 of this volume and the chapter on propagation in Vol. III.

Identification of Seedling Type (Nucellar versus Zygotic)

For breeding, rootstock use, and experimental work, it is important to be able to accurately distinguish zygotic and nucellar seedlings at an early stage. When the pollen parent is known and has morphological characteristics that clearly distinguish it from the seed parent, zygotic seedlings can be identified with some degree of accuracy. However, when the pollen parent is either unknown (open-pollination) or when its morphological characteristics are identical or similar to the seed parent, as in self-pollination or in "close" crosses, it becomes extremely difficult to distinguish between zygotic and nucellar seedlings. Even when the parents differ considerably in their characteristics, hybrids could be obtained that closely resemble the seed parent and might be identified as nucellar seedlings.

Furr, Reece, and Hrnciar (1946) and Nishiura, Matsushima, and Okudai (1957) used modifications of the rootstock color-reaction test in attempts to distinguish nucellar and zygotic seedlings in progenies obtained from known cross-pollinations. When both parents were very closely related or had similar color reactions, it was not possible to distinguish zygotic seedlings. When the parents had dissimilar color reactions, it appeared that the nucellar seedlings were often intermediate in color. However, Furr and Reece (unpublished) indicated that some seedlings identified as zygotic subsequently proved to be nucellar. Thus, this particular test does not appear to offer a basis for accurately distinguishing zygotic seedlings in progenies where it is also difficult to distinguish them on a morphological basis. Pieringer and Edwards (1965) used infrared spectroscopy to distinguish between nucellar and zygotic seedlings. Although it is a very sensitive technique, it may prove to have the same limitations as the colorimetric method. Pieringer, Edwards, and Wolford (1964) indicated that gas chromatography may be a usable technique for distinguishing between nucellar and zygotic seedlings.

STERILITY

The term fertility indicates the capacity for reproduction by means of gametes, ordinarily with fecundation. In the higher plants, complete sterility usually results in complete inability to reproduce by means of seeds. In plants having nucellar embryony, however, seedlessness requires not only true generative or sexual sterility, as just defined, but also what may be called nucellar sterility (inability to produce nucellar embryos).

Seed production in the citrus fruits may therefore be affected by factors, either genetic or environmental, which affect development of gametes, facility of pollination or fecundation, initiation of nucellar embryos, or survival of embryos. The following discussion is primarily concerned with generative sterility, which may or may not result in nucellar sterility. This subject requires rather full discussion here, for four main reasons: (1) the desirability of seedlessness of fruit for human consumption; (2) the desirability of asexual reproduction in the growing of rootstocks and in the establishment of nucellar selections; (3) the importance of sexual fertility in breeding; and (4) the relation of sterility to fruit-set and fruit-size (see pp. 298–300).

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Degree of Seediness in Cultivated Varieties

The average number of seeds per fruit, considered in relation to the number of ovules per ovary, gives a rough measure of fertility and sterility, although nucellar embryony complicates the interpretation of the evidence. There are characteristic varietal differences in seediness although much variation occurs (Moreira and Gurgel, 1941; Chapot and Praloran, 1955).

Complete seedlessness under all conditions is rare among citrus varieties. It may be characteristic of the Mukaku-Kishiu mandarin which is said to be a bud-variation strain of the seedy Kishui mandarin (Nagai and Tanikawa, 1928). Chapot and Praloran (1955) reported the Otaheite to be seedless. The Tahiti lime has been described as seedless (Hume, 1926; Uphof, 1931; Torres, 1932), but it is clear that occasional seeds can be produced (Traub and Robinson, 1937; Chapot and Praloran, 1955; Reece and Childs, 1962).

The Washington navel and various other varieties of navel orange rarely produce any seeds in ordinary culture because they entirely lack pollen. Since they produce some good embryo sacs, seeds can be produced by cross-pollination (Moreira and Gurgel, 1941; Majsuradze, 1951; El-Tomi, 1957*a*). Therefore, although under ordinary conditions of large-scale culture the common varieties of navel orange usually are entirely seedless, it is possible to use them as seed parents in crossing. Because of the absence of good pollen, however, they cannot be used as pollen parents.

The satsumas produce some pollen, but the fruits are usually seedless in the absence of cross-pollination. Pacher (1938) and Furusato (1951) indicate that it is possible to obtain adequate functional pollen for breeding under some environmental conditions. Using satsuma pollen, Reece (unpublished) has produced hybrid seedlings. With cross-pollination the satsumas ordinarily produce seeds considerably more readily than does the Washington navel (Miki, 1922).

Next in the scale of sterility after the satsuma come varieties which presumably all produce some good pollen, and which usually have seeds in many or all of their fruits, but in which the number of seeds is small or very small, the average usually being less than five, in some varieties commonly a little higher. Among such varieties are the Cadenera, Valencia, Boone, Shamouti, Enterprise, Hamlin, Verna, and Doblefina oranges; the Eureka, Lisbon, Berna, and Villafranca lemons; and the Marsh and other "seedless" grapefruits. In these varieties, only a very small number of the ovules usually develop into seeds, indicating a high degree of sterility.

Next to the few-seeded varieties may be grouped varieties which commonly have an average of from eight to ten or twenty seeds, including many of the mandarin varieties, the Paperrind, Parson Brown, Pineapple, and Ruby oranges and sour orange varieties. Even in these the numbers of seeds are very much smaller than the number of ovules that have been observed; for example, in the Shamouti orange Oppenheim and Frankel (1929) found about seventy ovules per ovary.

In other varieties, the number of seeds are still higher; in some varieties of grapefruit and shaddock (*C. grandis*), for example, the number may be as high as one hundred seeds per fruit.

It is therefore probable that most of the commonly cultivated varieties, if not all, have some degree of ovule or pollen sterility.

The seediness of some varieties can be greatly increased by cross-pollination. This effect can be produced in some varieties such as Shamouti orange (Oppenheim, 1929*a*, 1929*b*) and Kara mandarin (Soost, unpublished) that have a low percentage of viable pollen. In varieties that are self-incompatible (see p. 313), fruits set by self-pollination may be seedless, but fruit set by cross-pollination with compatible pollen will be seedy. Cross-incompatible varieties (see p. 313) could set seedless fruit when pollinated by incompatible pollen, but would produce seedy fruit when compatible pollen was used.

Kinds of Generative Sterility

With respect to the stage at which failure occurs, the cases of generative sterility may be divided into two main classes: gametic sterility and embryo (zygotic) abortion. Gametic sterility may be further divided into relative gametic sterility and absolute gametic sterility.

Relative Gametic Sterility (Self-Sterility and Cross-Sterility).—Self-sterility properly means self-incompatibility, or inability to form embryos by self-pollination although both the sperm cells and the egg cells are functional in suitable cross-pollination. Since the plants of one clone may all be considered as separate parts of one plant, the term self-sterility includes incompatibility in pollination between different plants of the same clone as between different trees of Clementine mandarin, for example.

The corresponding form of cross-sterility is cross-incompatibility, or inability to produce embryos with cross-pollination in certain kinds of matings, although the egg cells and sperm cells of the same plants are functional in other matings.

Self-incompatibility has been discovered in several varieties of *Citrus*. Nagai and Tanikawa (1928) found four apparently self-incompatible varieties among twenty-eight varieties tested. The Clementine mandarin has clearly been shown to be self-incompatible (Oppenheimer, 1948; Coste and Gagnard, 1956; Soost, 1956). The Orlando tangelo (Krezdorn and Robinson, 1958), the Minneola tangelo (Mustard, Lynch, and Nelson, 1956), the Robinson, Osceola, Page, Lee, and Nova mandarins (Reece and Register, 1961; Hearn and Reece, 1967) all appear to be self-incompatible. The Sukega grapefruit and hybrids of Sukega with Clementine, Orlando, and Minneola have all failed to set seedy fruits when self-pollinated although their pollen functions on other varieties and germinates *in vitro* (Soost, unpublished). Miwa (1951) reported the Hyuganatsu to be self-sterile but cross-fertile. Early reports indicated great differences in seediness of some pummelo varieties grown in different localities (Groff, 1927, 1930; Torres, 1932; Pope, 1934). Earlier, Boyle (1914) suggested that cross-pollination was the cause for the increased seediness in pummelos. More recent work definitely demonstrates the presence of self-incompatibility in at least several pummelo varieties (Nauriyal, 1952; Aala, 1953; Soost, 1964). Anjaneyula (1955) reported self-incompatibility for the lemon varieties Italian, Nepali Oblong, and Lucknow. Self-incompatibility is also indicated in *C. limettioides* Tan. (Singh and Dhuria, 1960) and in Malta lemon (Randhawa *et al.*, 1961).

Ikeda (1904–06) concluded from small-scale pollination tests that some varieties are cross-incompatible. Of the four self-incompatible varieties reported by Nagai and Tanikawa (1928), three also appeared to be cross-incompatible. Minneola and Orlando tangelos also appear to be inter-cross-incompatible (Mustard *et al.*, 1956; Krezdorn, 1960; Soost, unpublished). Data presented by Reece and Register (1961) indicated that Robinson mandarin may be cross-incompatible with Page and Osceola mandarins, although environmental conditions could have been responsible for the low set of seeds and fruit.

Orlando and Minneola tangelos are hybrids of Duncan grapefruit with Dancy tangerine (Reece and Childs, 1962). Robinson and Osceola are, in turn, hybrids of Clementine and Orlando, and Page is a hybrid of Clementine with Minneola (Reece and Register, 1961). The pattern of inheritance in these hybrids and in the hybrids of Sukega listed above indicates the presence in *Citrus* of a set of incompatibility alleles. On this basis, it would be predicted that all progeny resulting from intercrossing self-incompatible varieties, such as Clementine and Orlando, would also be self-incompatible. The presence of such an incompatible system in an insect-pollinated genus is not surprising and may be more widespread in *Citrus* and related genera than has generally been realized.

Whether cross-sterility due to general unlikeness of parents occurs at all among ordinary forms of *Citrus* is uncertain (see chap. 5). There is some evidence of cross-sterility between forms with different chromosome number (see chap. 5). Toxopeus (1931) discovered a peculiar form of partial cross-sterility related to the length of the style; when certain varieties which have very short styles are crossed reciprocally with ordinary long-styled varieties, the pollen of the short-styled varieties is comparatively ineffective in producing seeds, presumably because the pollen tubes grow too slowly or stop too soon. Torres (1938) concluded that "some varieties of different species" seem to be intersterile, but that this is due to genetic differences, not to likeness in incompatibility genes, "since citrus [forms] are generally self-fertile." However, the accumulating evidence suggests that self-incompatibility may be involved (see above).

Absolute Gametic Sterility.—Male or pollen sterility consists of inability to produce functional pollen, that is, pollen with sperm cells capable of fecundating egg cells. Female or embryo-sac sterility consists of inability to produce embryo sacs with egg cells capable of development into embryos. Absolute gametic sterility may be roughly classed as genetic or nongenetic in origin. In the former, the hereditary constitution is such that the plant or variety is sterile (partially or completely) under conditions which are favorable to fertility in other genetic types; absence or abnormality of the reproductive organs or cells is characteristic of the variety affected. The Washington navel orange is an example of genetic sterility. Nongenetic sterility is due to environmental causes which are comparatively unfavorable to the reproductive processes. Iwamasa and Iwasaki (1963) associated low temperatures with the reduction of pollen fertility in *C. aurantifolia.* Brieger and Gurgel (1941) reported that certain rootstock varieties decreased pollen fertility.

Obviously, sterility may be both characteristic of a variety and variable in that variety according to the environment. Nakamura (1943) found temperature influenced pollen fertility in varieties that had some degree of sterility under normal conditions.

In some varieties, part of the flowers have abortive pistils whose development has been prevented, or interrupted in various stages, perhaps by competition of other flowers or buds for food material, or by production of inhibitors. The flowers of a fertile citrus variety may be either (1) both perfect or hermaphroditic and staminate or male (polygamomonoecious), or (2) regularly perfect (Toxopeus, 1931; Uphof, 1932; Oppenheimer, 1935; Minessy, 1954; Singh and Dhuria, 1960; Randhawa *et al.* 1961). In the former class, which includes *Citrus limon, C. aurantifolia,* and *C. medica,* and in forms that appear to be closely related to these species, the pistil is regularly underdeveloped or absent in a large part of the flowers. In the latter class, which includes *C. sinensis, C. grandis, C. paradisi,* and *C. reticulata,* abortion of the pistil is much less common. However, it may occur frequently among the later-opening and weaker flowers. The proportion of staminate flowers is highly variable, according to the variety and the growth conditions. Scaramella-Petri and Strigoli (1958) reported that low temperatures (0°C to 10°C) during flower formation increased the number of aborted pistils in *Poncirus trifoliata.* In varieties with comparatively few perfect flowers, these flowers are likely to set fruit in much higher proportions than in varieties in which the flowers are regularly perfect.

The stamens, unlike the pistil, show very little tendency to general failure of development, although in one variety of navel orange which produces some pollen (Shamel, 1918) part of the stamens are often abnormal, being more or less petaloid. Hybrids of satsuma mandarin obtained by Iwamasa (1966), using several pollen parents, did not develop normal anthers. Anjaneyula (1953) reported the transformation of other floral parts, including stamens, into petals in Sathgudi orange, kumquat (*Fortunella* sp.), and lemon. Imperfect development of part or all of the pollen mother cells or the pollen is common, however. The ability of pollen to produce fecundation is probably better indicated by its germination in suitable culture media than by its appearance under the microscope. However, germination *in vitro* is not an absolute measure of the ability of the sperm to accomplish fecundation. Techniques for *in vitro* germination have been used on many *Citrus* varieties by many investigators (Nagai and Tanikawa, 1928; Herrero-Egaña and Labiada, 1935); Moreira and Gurgel, 1941; Oppenheimer, 1948; Plaut, 1947; Pereau-Leroy, 1950; Zacharia, 1951; Nauriyal, 1952; Yatomi, Koga, and Uchida, 1952; Mustard *et al.*, 1956; Krezdorn and Robinson, 1958; Damigella and Squillaci, 1959; Singh and Dhuria, 1960; Ozsan, 1961; Randhawa *et al.*, 1961; Singh and Randhawa, 1961). Most procedures have used 20 per cent sucrose solutions at 20°C to 25°C. Resnick (1958) indicated varietal differences in response to the concentration of sucrose. With most varieties that he tested, germination was best in 30 to 50 per cent sucrose, but Shamouti germinated best in 15 per cent. With lemon and citron varieties, Randhawa *et al.* (1961) obtained the best germination in 15 per cent sucrose. Germination on agar rather than in liquid media has also been satisfactory. Various materials have also been added in attempts to increase germination. Resnik (1956) reported increased germination from additions of 2,4-D, thiamine, indolebutyric acid, or boric acid. Singh and Randhawa (1961) obtained increased growth of pollen tubes with additions of gibberellic acid, but the percentage of germination was decreased. Large differences in the germination percentage for a single variety have been reported by various investigators. In addition to possible errors in varietal identification, a lack of uniformity in germinating conditions and methods of handling the pollen is indicated.

In some kinds of plants, certain genes or combinations of genes are lethal for the development of the particular pollen grains or embryo sacs, or both, which receive them, so that these pollen grains or embryo sacs do not produce viable gametes. Also, possession of a particular gene constitution, sometimes of one particular kind of gene, may prevent the development of stamens or pistils, or of their reproductive tissues, throughout the plant; the sterility of the Washington navel orange is of this sort, since its pollen mother cells degenerate before reduction division.

Irregular chromosome reduction, leading to presence of extra chromosomes or lack of a part of the complete normal set, may lead to gametic sterility or embryo abortion. This cause of sterility is mainly responsible for the nearly complete sterility in citrus triploids, and must greatly reduce the fertility of tetraploids. Irregular reduction of chromosomes often occur in F_1 plant hybrids from wide crosses; much irregularity was found by Longley (1925) in the Eustis limequat. Some diploid varieties of *Citrus* show evidence of irregular reduction (see p. 294).

Even with regular reduction, F_1 plant hybrids from wide crosses often produce unbalanced sets of chromosomes, sets derived partly from each parent often being seriously incomplete or otherwise deleterious.

Defective Pollen Development (Male Sterility).—In the Washington navel orange, casual inspection suffices to show that no pollen is shed, and that the mature anthers, at the time of the opening of the flower, are cream in color, strikingly unlike the bright yellow which is produced by the pollen in fertile anthers. At the time when the normal anthers have just shed their pollen, the Washington navel anthers, usually unopened, are shriveling and changing to a dingy brownish color.

Microscopic examination at earlier stages shows that the sporogenous tissue degenerates before the time of the first meiotic division (Webber, 1894, 1930, Osawa, 1912). The anthers usually develop well-differentiated pollen mother cells and tapetum before development stops (fig. 4–12), although sometimes degeneration starts at an earlier stage, in the primary sporogenous cells. If pollen mother cells have been produced, they degenerate without cell division, some appearing to form two or three nuclei within the original cell wall, and somewhat later the tapetum also degenerates (fig. 4–12). Remnants of pollen mother cells and tapetal cells may sometimes be found in the mature anthers (fig. 4–12) but no mature pollen is produced (Nakamura, 1934, 1943; Moreira and Gurgel, 1941). The Washington navel therefore is completely male-sterile, and is incapable of sexual reproduction except as a female parent in crosses.

Occasional reports of well-developed pollen in this variety probably relate to distinct genetic types rather than to normal pollen development occurring under exceptional conditions in the Washington navel itself. The pollen-bearing Rufert orange is a selection found among nucellar seedlings of a pollenless navel variety (Ruvel) at Riverside, California (see chap. 5).

Nakamura (1934) found that in several varieties the pollen mother cells degenerate about as in the Washington navel. These are the Thomson, Navelencia, Surprise, and Pyriform oranges (of which the first two, at least, were derived by bud variation from the Washington navel), and the Tahiti lime. Uphof (1931) also observed this behavior in the Tahiti lime. Iwamasa (1966) reported the same degeneration in hybrids of satsuma mandarin with *Poncirus trifoliata*.

Satsumas are often completely male-sterile, producing very low percentages of well-developed pollen. Osawa (1912) observed that most of the pollen grains degenerated at various stages of development (fig. 4–12, *F*), only a few pollen grains appearing normal at anthesis. Longley (1925) and Ozsan (1961), however, found more than 50 per cent of the pollen grains appearing normal. Several investigators (Osawa, 1912; Nagai and Tanikawa, 1928; Zorin, 1936; Pereau-Leroy, 1950; Ozsan, 1961) have also been able to obtain germination *in vitro* and Reece (unpublished) has produced hybrids using satsuma pollen.

The average number of pollen mother cells in a satsuma loculus is comparatively small; the number is highly variable, and some microsporangia produce none at all. Osawa (1912) found that the pollen mother cells sometimes degenerate, but that in most cells the reduction divisions

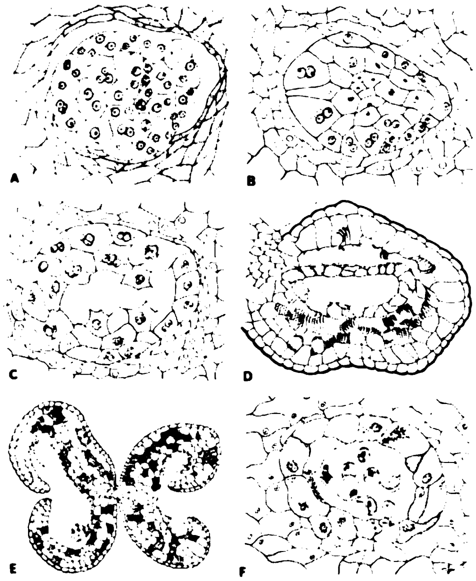

Fig. 4–12. Degeneration in the anther in sterile varieties. *A-E*, Washington navel orange; *A*, cross-section of a microsporangium, apparently normal; *B*, *C*, *D*, same, later showing degeneration; *E*, cross-section of mature anther, destitute of pollen; *F*, satsuma, microsporangium, pollen grains mostly degenerated. (Redrawn from Osawa, 1912.)

appear normal. Nakamura (1929), however, found irregularities of meiosis to be common, and this suggests the presence of a chromosome constitution capable of causing pollen sterility.

In the very few-seeded Shamouti orange, Oppenheim and Frankel (1929) found that the reduction divisions in the pollen mother cells, and the formation of the microspores, usually appeared normal. Zacharia (1951) reports about 38 per cent of the pollen is shriveled and pale, about 23 per cent is normal appearing, and about 39 per cent is normal and large. Germination results (Oppenheim and Frankel, 1929; Oppenheimer, 1935; Zacharia, 1951; Ozsan, 1961) have been variable but usually less than 10 per cent of the pollen has germinated; Ozsan (1961) obtained a maximum of 27.9 per cent. Zacharia (1951) found that pollen-tube growth in the style was good but suggests that the ovules may not be reached, resulting in fruit set by induced parthenocarpy (see p. 298).

High percentages (40 to 60 per cent of empty pollen have been reported for Valencia orange (Longley, 1925; Uphof, 1934; Moreira and Gurgel, 1941). Cameron *et al.* (1960) obtained increased seed set from cross-pollination, indicating that low seed production is partly due to lack of functional pollen.

Marsh grapefruit has been found to have from 5 to 15 per cent well-developed pollen (Longley, 1925; Friend *et al.*, 1939; Moreira and Gurgel, 1941; Ozsan, 1961). Ozsan obtained a maximum germination of 3.2 per cent. Other seedless grapefruit also have low percentages of well-developed pollen; Ozsan found about 8 per cent in Thompson.

Nakamura (1943) found rather large proportions of defective pollen in several varieties of *C. limon.* Moreira and Gurgel (1941), Pereau-Leroy (1950), and Ozsan (1961), also found much defective pollen in the Eureka variety and others. Nakamura (1943) found many abnormal tetrads and rather high frequency of univalents in Eureka lemon. The frequency of univalents and abnormal tetrads increased as the temperature was reduced from 19°C to 10°C. Chen (1944) also reported abnormal tetrads in Eureka. Naithani and Raghuvanshi (1958) found inversions in Italian long and univalents in Italian oblong. They also note "fusion of pollen mother cells to form giant binucleate cells." However, Randhawa and Choudhury (1960) reported high pollen germination and very few meiotic abnormalities in the seedless varieties Malta, Nepali Oblong, and Seedless lemon, or in the seedy variety Genoa. It seems clear that much pollen sterility, perhaps caused by meiotic abnormalities, occurs throughout *C. limon.*

Moreira and Gurgel (1941) found several varieties of oranges that have high proportions of defective pollen. In addition to the varieties already discussed, they include Lima, Pera, Cadenera, and several others. All of these varieties are low in number of seeds.

Nakamura (1943) found many other varieties and species, particularly those that appear to be taxonomically related to *C. limon,* that show a high degree of pollen sterility. Among these are Otaheiti, rough lemon, Bouquet sour orange, and *C. junos* 'Kizu.'

Pollen sterility of varying degree has therefore been found throughout the genus *Citrus,* culminating in the conditions found in varieties where no functional pollen is produced.

Defective Embryo-Sac Development (Female Sterility).—Osawa (1912) found that both in the satsuma and in the Washington navel orange the megaspore mother cell or the embryo sac very often degenerates. Sections of the ovule of the Washington show that its undivided embryo-sac mother cells often become shrunken with degenerating cytoplasm, which stains deeply. More often, however, with both varieties, such changes are not noticeable until after the row of four megaspores has been formed (fig. 4–13, *A*) or even until one of the spores has divided to form the embryo sac (fig. 4–13, *B*). A few embryo sacs reach full development and contain egg cells capable of fertilization, as is proved by the production of

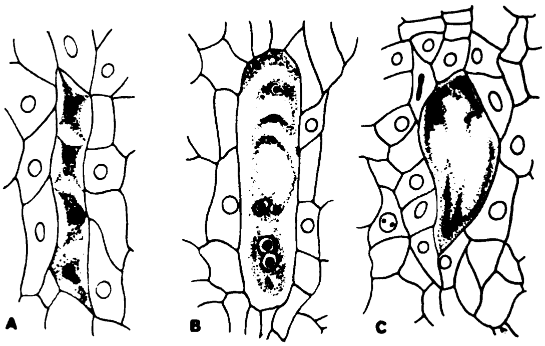

Fig. 4–13. Degeneration in the ovule of sterile varieties. *A*, Washington navel orange, megaspores all degenerated; *B*, same, embryo sac degenerating; *C*, satsuma, embryo sac degenerated. (Redrawn from Osawa, 1912.)

seeds and of seedlings, including occasional hybrids, by cross-pollination.

Since the other tissues of the ovule develop normally, the degeneration is not a matter of generally poor development of the ovule, but must depend on some more specific tendency to poor development of the reproductive tissue. Neither is the abnormality a matter of irregular chromosome reduction, since degeneration is often noticeable in the prophase of the first meiotic division. It is presumably due to the same general genetic cause as is the degeneration of the pollen mother cells in the same varieties. In this connection, it seems significant that in both anther and ovule the defective development is more extreme in the Washington navel than in satsumas.

Apparently very few functional ovules are formed in the Tahiti lime. Although Uphof (1931) reported that embryo sacs are not formed, occasional viable seeds are produced (Reece and Childs, 1962). Bacchi (1940) reported the Tahiti lime to be a triploid, but Reece and Childs (1962) indicate the chromosome number to be diploid.

Although the low percentage of functional pollen is a major factor in the low seed number of the Shamouti orange, the embryo sac is absent or abortive in a large part of the ovules (Oppenheim and Frankel, 1929; Zacharia, 1951). Considerable female sterility is also indicated in the Valencia orange by the low seed set obtained from cross-pollination (Cameron *et al.*, 1960). Undoubtedly, other orange varieties that have few seeds, such as the Cadenera, also have much female sterility as well as pollen sterility.

The low seed set obtained from cross-pollination indicates a high degree of female sterility in the Marsh grapefruit. Other seedless varieties of grapefruit also must possess much female sterility.

Embryo Abortion

Functional gametes may be produced, fecundation may occur, and yet the fecundated egg may be unable to develop into an embryo capable of germination. If no embryos are able to develop, complete zygotic sterility results. This sterility, like gametic sterility, may be either genetic or nongenetic in origin. The fate of the fecundated eggs, however, does not tell the whole story with respect to embryo production in *Citrus* and its nearest relatives, since many varieties are capable of producing embryos by an asexual process. The relation of nucellar embryony to sterility must therefore be considered.

The occurrence of empty seed coats and nearly empty seed coats suggests embryo abortion. Seeds which produce only nucellar seedlings indicate failure or suppression or possible absence of zygotic embryos.

Relation of Nucellar Embryony to Sterility

Evidence already discussed indicates that absence of pollen usually, if not always, prevents the development of nucellar embryos. Complete male sterility, without cross-pollination, therefore results in seedlessness. Also, since the nucellar embryos develop in the embryo sac, presumably such embryos cannot be produced unless the embryo sac is well developed; and it is probable that fecundation of the egg or union of the polar nuclei and sperm is usually necessary. On the other hand, since certain varieties produce nucellar seedlings mainly or exclusively, and in other varieties many seeds produce nucellar seedlings only, it is probable that abortion of the zygotic embryo does not necessarily prevent the development of nucellar embryos. In brief, then, it is probable that gametic sterility usually necessitates nucellar sterility, but that abortion of the gametic embryo does not.

Sterility of Diploid First-generation Hybrids

The complications introduced by nucellar embryony and by the prevalence of sterility in ordinary varieties make it difficult to obtain adequate evidence on the relation of known hybridization to sterility. In any adequate attempt to evaluate the fertility or sterility of particular selections, it is necessary to consider four features, namely, fruitfulness, seediness, number of embryos per seed, and the relative proportions of zygotic and nucellar seedlings. The possibility that nucellar embryos may develop in spite of complete gametic sterility must also be kept in mind.

First, it is certain that many fertile hybrids are produced from interspecific and even intergeneric hybrids. From open pollination of fifty-six recent F_1 hybrids between four varieties of *Citrus* and *Poncirus trifoliata* (Cameron and Baines, unpublished) mainly zygotic seedlings were obtained from forty-three, and mainly apparent nucellar seedlings were obtained from thirteen others. Altogether, this indicates a high degree of female fertility in these intergeneric hybrids. Pollen fertility has not been checked. In many hybrid populations between various *Citrus* species at Riverside, California, most individuals have a high degree of fertility as indicated by fruit set, seed number, and pollen viability. Often the proportion of evident nucellar embryos is very high, making it impossible to determine generative female fertility. However, many individuals produce some evident zygotic seedlings. It is also clear that the amount of fertility is inherently variable among F_1 hybrids from the same cross. Some hybrids involving parents that exhibit much sterility are highly sterile, exhibiting degenerated pollen, low seed production, and poor set. Other individuals are decidedly seedy, although most of their seedlings are of nucellar origin. Finally, it is obvious that the presence of nucellar embryony is involved in producing the appearance of female sterility. In populations segregating for polyembryony (see chap. 5), monoembryonic individuals exhibit a high degree of generative fertility, but zygotic seedlings are low in number or lacking in progeny from polyembryonic individuals. Such a close correlation between generative sterility and polyembryony is not likely. Many individuals that produce nucellar seedlings almost exclusively also are highly pollen fertile.

Zygotic progeny produced from self-pollination or cross-pollination of many closely related varieties are mainly very weak, some never producing flowers. However, some individuals equal or surpass either parent in vigor of growth and seem fully fertile. Hybrid progeny from crosses within the species *C. reticulata* are generally more vigorous than progeny from crosses within the other species to which most of the cultivated varieties are assigned.

The Horticultural Importance of Sterility

The one general important horticultural advantage of sterility lies in the resulting absence or scarcity of seeds. Because of the location of the seeds and the usual methods of using the fruits, seedlessness is very desirable from the viewpoint of the consumer. In California, all of the main commercial varieties are few-seeded or seedless, and this characteristic unquestionably determined the adoption of the Marsh grapefruit. Fahey (1940) reports that seeded varieties of grapefruit in Trinidad are practically not salable since the introduction of seedless varieties. Low seediness has also been an important factor in the adoption of the Washington navel, Shamouti, and Cadenera oranges, the Tahiti lime, and satsuma mandarin. The undesirable increase in seeds following cross-pollination of some varieties has led to suggestions that these varieties should be isolated from other varieties. However, the lack of cross-pollination results in severe reductions in yields in at least some of these varieties (Clementine, Orlando, Minneola, and pummelo varieties).

As previously discussed (p. 298), failure to set seeds often tends to prevent or reduce the setting of fruit. Sterility therefore tends to be a disadvantage with respect to yield. It may in some varieties, however, be advantageous by preventing excessive setting of fruit and consequent small size of fruit, overloading of trees, and alternate bearing. Washington navel orange and satsuma mandarin exhibit much less alternation of yield than do mandarin varieties which have many seeds.

Embryo abortion and perhaps gametic sterility may affect the degree to which a stock variety "breeds true" by nucellar embryony. For rootstock use, it is, of course, desirable to have uniformity in the seedlings. If sterility limits the number of seeds produced, it can limit the usefulness of a variety that would otherwise be a valuable rootstock.

Low generative fertility is obviously a serious hindrance to the use of a variety in hybridization. Complete or even a high proportion of pollen sterility is especially troublesome when, as in the navel oranges and the satsuma mandarins, the proportion of nucellar embryos is very high, such varieties are difficult or impossible to use as pollen parents, and when they are used as seed parents their seedlings include only small proportions of hybrids. For the search for usable variations among nucellar offspring, "nucellar fertility" is necessary, and perhaps, therefore, some degree of gametic fertility also. The unfavorable effect of seedlessness on the setting or retention of fruit is also a hindrance in the production of usable new seedless varieties. However, the desirability of having seedless varieties has led to the breeding of triploid varieties (see chap. 5).

CYTOLOGICAL METHODS FOR CITRUS

Acetocarmine or propionocarmine smears have been much used for chromosome counts in pollen mother cells of citrus, although differentiation is often poor even with fresh anthers. Premordanting with iron alum (4 per cent) improves differentiation but the results are still not completely satisfactory. With fresh anthers, the substitution of orcein for carmine gives much better differentiation (see fig. 4–6, p. 294). The use of a 0.25 per cent, or even lower, solution in 45 per

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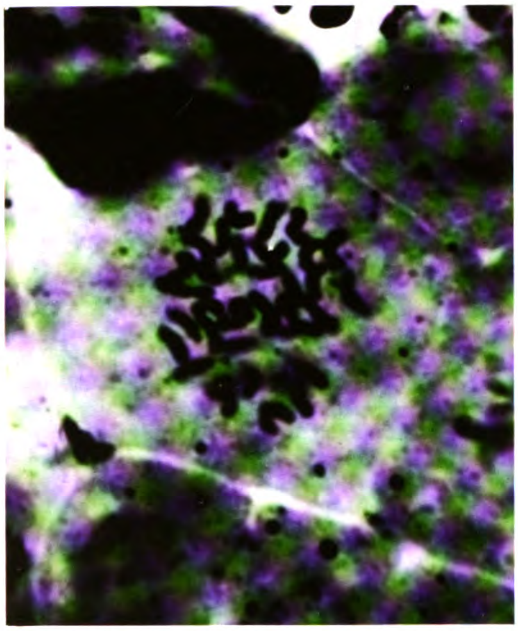

Fig. 4–14. Chromosomes of tetraploid *Poncirus trifoliata* in a root-tip smear. Acetocarmine stain following pretreatment with coumarin and acid hydrolysis. (Slide by S. K. Mukherjee.)

cent acetic acid has given the best results. It may be necessary to leave the slides for 24 hours or longer before full staining develops. With killed and fixed anthers, neither carmine nor orcein has been completely satisfactory.

Individual buds may show a wide range of stages in the anthers, seldom having any very large part of the pollen mother cells at metaphase or anaphase of either division. With some varieties, especially good smears can be obtained from buds cut transversely, held by the base, and wiped on a dry slide. Smashing the anthers is also satisfactory, although debris can be a problem. The length of bud at which the reduction divisions have been found in diploids at the Citrus Research Center, Riverside, California, has been about 2 to 3 mm for small-flowered varieties, 3.5 to 5 mm for sweet orange and grapefruit, and 5 to 6 mm for lemons. Tetraploids have slightly longer buds than the corresponding diploids.

Randhawa and Choudhury (1960) used the method of Tijo and Levan (1950) for preparing root-tip squashes. Sharma and Bal (1957) pretreated root tips with aesculin, coumarin, or paradichlorobenzene. Mukerjee and Cameron (1958) reported that best results were obtained with alcohol-acetic fixation followed by coumarin treatment prior to squashing and staining (fig. 4–14). Preliminary tests with a pretreatment of 8-hydroxyquinoline sulphate followed by hydrolyzing in 1*N* hydrochloric acid and staining with aceto-orcein have produced promising results at the Citrus Research Center.

The chromic-acetic-formaldehyde fixer is very satisfactory for chromosome study in citrus root tips, for staining either with crystal violet (Bacchi, 1940) or with hematoxylin (Krug, 1943). Tertiary butyl alcohol is most often used for dehydration. Sharma and Bal (1957) also used chromic acid and formalin or platinic chloride and formalin as fixatives. Staining in crystal violet is preceded by either overnight bleaching in 95 per cent ethyl alcohol and water (1:1) or premordanting in 1 per cent chromic acid for 24 hours, depending on the fixative used.

In sectioning embedded material for cytological examination, crystals of glucoside may be troublesome; Bouin's fixer and Flemming's stronger fixer have therefore been recommended. Treatment with alcoholic potassium hydroxide may also be used. Heidenhain's hematoxylin has been widely used either alone or followed by other stains (Oppenheim and Frankel, 1929; Ruggieri, 1935; Nasharty, 1945; Banerji, 1954).

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CHAPTER 5

Genetics, Breeding, and Nucellar Embryony

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THIS CHAPTER summarizes evidence on heritable and nonheritable variation in citrus from studies carried out principally during the past seventy years. Considerable description of the physiological effects of nucellar embryony is included. Mutation, bud variation, and chimeral relationships, all prominent in many citrus varieties, are discussed in some detail. The goals and results of some citrus breeding programs are described.

In Chapter 10, Volume I, of the first edition of *The Citrus Industry* (Webber and Batchelor, 1943), A. D. Shamel presented extensive data on bud variation and bud selection in citrus. Certain portions of his material are incorporated in this chapter.

GENERAL ASPECTS OF CITRUS VARIATION

Variation in living organisms may be either genetic or nongenetic. Differences such as those distinguishing the Valencia orange from the Washington navel orange are inherent in the genotypes of the trees; thus, trees budded from a Valencia regularly remain Valencia in type. On the other hand, Valencia trees are seldom phenotypically identical, although most of the differences among them are not heritable. Nonheritable variations are usually produced by differing conditions in the environment such as climate, nutrition, and disease.

In vegetative propagation by such means as budding, grafting, or nucellar embryony, the genetic constitution of the progeny is usually identical to the parent because the somatic cells of a plant commonly have the same constitution. Sexual reproduction, in contrast, recombines the genes and usually produces numerous genetic differences between parent and progeny.

During the development of a plant or animal, differentiation of tissues and organs systematically occurs despite the genetic similarity of somatic cells. Development is principally controlled by the actions of genes and gene products; it depends also on the interrelations among parts, such as position and arrangement of cells. Recent studies indicate gene actions are highly selective in their timing and localization, so that critical processes are induced or repressed in orderly sequence.

Heritable variations are the basis of genetics and breeding, but their analysis is greatly complicated by the problem of distinguishing them from nonheritable ones. In citrus, climatic factors are very effective in producing modifications among like parts of a tree, such as individual fruits or leaves. Light, temperature, and wind are of particular importance. Both environment (fig. 5–1) and the long period of juvenility following seed reproduction can markedly modify characteristics important in citrus breeding, such as size, shape,

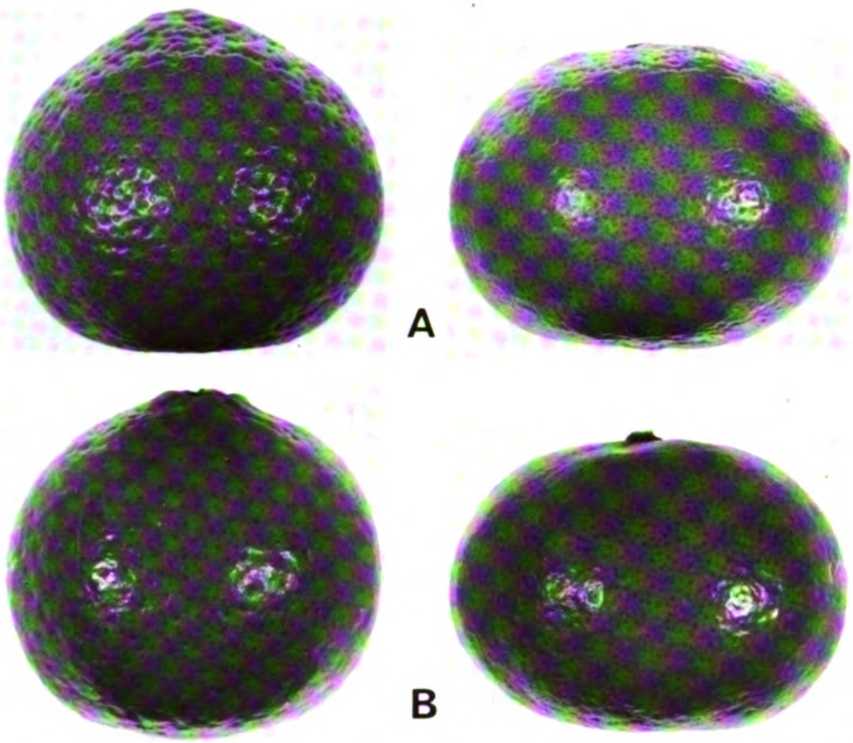

Fig. 5–1. Effect of environment on fruit shape. *A*, Kara mandarin fruits; *B*, a King × Dancy hybrid. Fruits on the right were produced near Tustin, California, in a coastal, subtropical climate; those on the left were from Riverside, California, some 30 miles farther inland. Differences in shape and roughness can also be affected by other factors.

yield, and flavor of the fruit. Adequate evaluation of the genetic characters of a new variety, for example, requires that it be observed in various environments over a long period of time.

Modifications due to virus infection have become of primary importance in citrus. The number and variability of virus symptoms often make it very difficult to define the effects of other factors, genetic or nongenetic. This is especially true with respect to the analysis of age changes, which are discussed below.

VARIATION DUE TO AGE

General Biological Considerations

Age changes must be considered with respect both to individuals and clones. Changes in individual organisms accompanying changes in age are widely recognized and have been the subject of many studies. Because of the nature of the animal body, with its usually determinate growth and highly differentiated parts, much more is known concerning age changes in animals than in plants.

Minot (1908) concluded that normal cell differentiation in animals tends to go too far, with injurious results. Bidder (1932) considered mechanisms limiting final size in vertebrates at least indirectly responsible for senescence. Later workers have demonstrated many conditions contributing to the aging process. Comfort (1964), in a comprehensive review of animal senescence, concluded that no single explanation is widely applicable. He defined senescence as "a change in the behavior of the organism with age, which leads to a decreased power of survival and adjustment." Some important characteristics usually associated with aging (and at least partly verified by experiments) are cessation of growth, a high degree of differentiation, and sometimes the speeding up of metabolic rates.

Age changes in plants have proved even more difficult to explain than those in animals. As reviewed by Sax (1962), various species show great differences in their life spans, ranging from thousands of years in *Sequoia* and certain pines to a few weeks in some annuals. In annual plants, death systematically follows flowering and seed maturation, although it can often be delayed by the prevention of these stages. Thus, Leopold, Niedergang-Kamien, and Janick (1959) showed that soybean plants with their flowers removed lived nearly three months longer than plants which matured seed. But Lockhart and Gottschall (1961) found that the Alaska pea, despite deflowering, finally differentiated an apical node that no longer contained a vegetative meristem.

In perennials propagated asexually, there has long been uncertainty as to the meaning of changes occurring with age. Knight, as early as 1795, held that clonal senescence did occur. Other early workers concluded that there is no unavoidable aging process (Möbius, 1897; Winkler, 1920). Yet Molisch (1938), after extensive consideration of the evidence, concluded that changes due to old age occur in clones, and that sexual reproduction produces a rejuvenation which does not result from artificial vegetative propagation.

In considering the problem of age changes in citrus, we should differentiate between early effects of juvenility in seedling selections and possible old-age effects occurring after reproductive maturity has been reached. It is clear that a juvenile period exists as a part of the life cycle of many plants, including citrus. Robbins (1957*a*), Brink (1962), Sax (1962), and Doorenbos (1965) have reviewed the subject. Differences between juvenile and adult leaf shape are marked in certain eucalyptus and juniper varieties. In the beech, old trees shed their leaves in autumn, but young seedlings retain them in a dried condition. The trunk region and root system remain juvenile after the upper parts of the tree have passed into the leaf-shedding phase. In apples, leaves of one-year-old seedlings are thin and lack pubescence (Stoutemyer, 1937); these characteristics change as the seedlings grow older. However, studies by Wellensiek (1952) showed that with certain mature woody plants, adventitious shoots produced on disbudded stems displayed some juvenile characters.

The English ivy *(Hedera hèlix)* provides evidence for contrasting juvenile and adult stages, the latter being sometimes reversible (Robbins, 1957*b*; Stoutemyer and Britt, 1961). The juvenile form, which is a trailing vine without flowers, can change abruptly to an upright form bearing inflorescences. Seedlings always first display the juvenile condition. Reversion from adult to juvenile form has been observed following pruning, grafting of adult scions onto juvenile stocks, and treatment with gibberellic acid.

Changes Associated with Age in Citrus

In citrus, juvenile characters are very prominent and often persist for a long time. They include thorniness, vigorous and upright habit of growth, slowness to fruit, alternate bearing in early years, and physical differences in fruit characters. In early years, the most striking character is thorniness. Swingle (1932) emphasized thorniness in discussing the marked rejuvenation which occurs in citrus after seed reproduction.

Thorniness.—Most species and varieties of *Citrus* produce thorns, which are imperfectly developed branches (Uphof, 1935*a*). Occasionally a vigorous thorn shows its true nature by producing leaves and even flowers, and rarely a thorn may be replaced by an ordinary leafy shoot which develops at the same time as the thorns of neighboring nodes (fig. 5–2). This shows that thorns are rudimentary lateral branches which develop during the same growth cycle as the stem that bears them, whereas ordinary lateral branches develop in a later growth cycle. Very vigorous trees and shoots are especially likely to be thorny.

On ordinary trees of most old budded varieties, thorns are few and small, although in the Lisbon lemon, for example, they are often pronounced enough to cause annoyance. Young nucellar seedlings, grown from such old varieties, show thorniness which is many times as great as on the parent tree. The immediate budded prog-

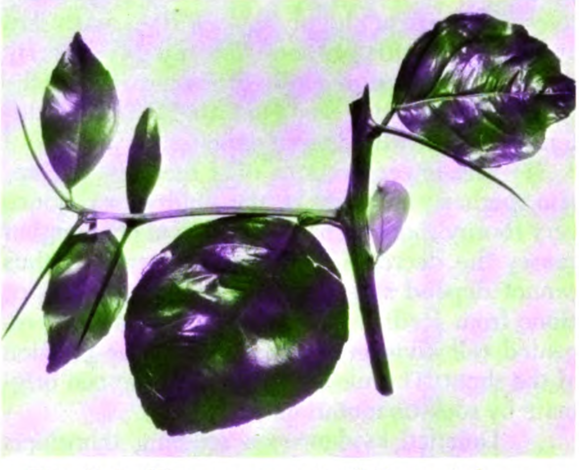

Fig. 5–2. Vigorous sprout of Paperrind orange, with one thorn replaced by a nearly normal shoot which developed at the same time as the thorns at nearby nodes.

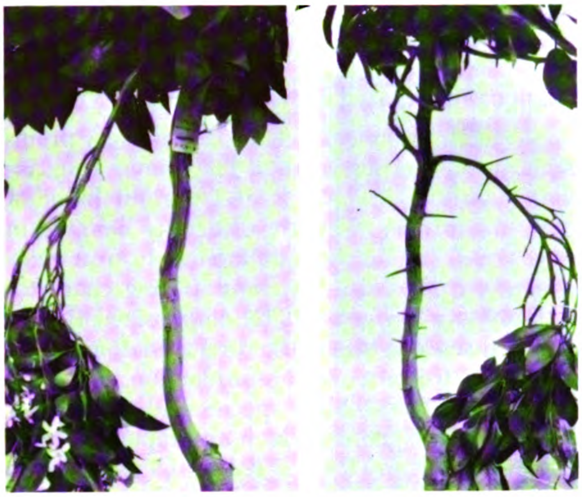

Fig. 5–3. Nursery trees of Kara mandarin, budded from the same parent tree. Left, tree with few thorns, budded from a high, thornless shoot; right, thorny tree budded from a low, thorny shoot. The Kara variety was then 13 years old from seed. Tree on left had many flowers; the one on the right had few.

eny of these seedlings may also be very thorny. The thorniness of young zygotic seedlings and their budded progeny is variable. It is usually high, however, and on the average is similar to that of young nucellar individuals from the same parent varieties. The following considerations apply in general to both kinds of plants.

As thorny seedlings become older, much of the new growth, especially high in the top, is decidedly less thorny. If bud sticks are cut from top shoots low in thorns, they are likely to produce trees which are not highly thorny. In early studies at Riverside, this procedure often gave nearly thornless trees within ten to fifteen years from the germination of the seed, but the reduction differed greatly with different varieties.

The lower portions of a seedling tree retain, perhaps indefinitely, the ability to produce very thorny shoots. The physiological change that causes the decrease of seedling thorniness thus cannot depend simply on the age of the tree or clone from seed; it may be favored rather by repeated cell division and perhaps by the position of the shoot. Thornlessness perhaps also can originate by somatic mutation.

Detailed evidence on seedling thorniness was obtained by Frost (1938) in breeding studies beginning about 1915. The correlation in relation to certain parental types was marked. With the satsuma mandarin, which is low in thorns, young nucellar seedlings and their budded progeny usually had little thorniness, whereas in sweet oranges thorniness was high and especially persistent. Certain hybrids of the Bouquet sour orange seemed to produce no thorns at all. Many hybrid and nucellar seedlings from various species were grown in crowded plantings, at 2- by 5-foot spacing, and were repeatedly pruned back severely for about ten years. Most of the seedlings were originally very thorny, and at the end of the period thorniness of the new growth seemed undiminished. This was true also of budded trees from some of the seedlings, handled in the same way.

An orchard planting at wider spacing was later made with other budded trees from these seedlings; this planting was not cut back appreciably. After a few years, the newer growth, especially on high branches, was much reduced in thorniness. When a second budded generation was propagated from some of these trees, after three to five years, budwood selected for thornlessness gave less thorniness in the nursery than had the earlier budding. A few original hybrid seedlings, first held in close planting until sixteen years old, were also severely pruned and set in the orchard. In the first years thereafter, these trees were much more thorny than second-budded-generation trees earlier derived from them. Similar trends in thorniness have since been generally observed by citrus breeders.

Figure 5–3 shows the contrasting degrees of thorniness in nursery budlings of Kara mandarin, resulting from selection of thornless and thorny budwood from the same tree. Figure 5-4 illustrates low thorniness in nursery trees budded from Minneola tangelo, contrasted with high thorniness on budlings from a recent nucellar seedling of Minneola.

Frost (1952) contrasted thorniness in old budlines and recent nucellar selections of fifteen citrus varieties. Nursery budlings were propagated in 1930 from old orchard trees and from the first budded generation of selections about fifteen years of age from seed. The seedling selections had usually arisen from the same trees which served as old budline sources. Budwood from the seedling selections was taken from both high and low positions. At three years of age, resulting trees from almost all of the seedling selections were much more thorny than those from the old budlines. Trees from low budwood were more thorny than those from high budwood. Some of the data are shown in table 5–1.

Table 5–1

THORNINESS AND FRUITING OF THREE-YEAR-OLD CITRUS BUDLINGS FROM OLD BUDLINES AND YOUNGER NUCELLAR SEEDLING SELECTIONS*

Variety	Budwood Source Within Clone	Relative Thorniness of Budlings†		Average Number of Fruits Per Budling
		Nucellar vs. Old Budline	Low vs. High Budwood	
Eureka lemon	Old budline			8.7
	Nucellar	++	++	0.2
Marsh grapefruit	Old budline			8.2
	Nucellar	++	+++	0
King mandarin	Old budline			18.0
	Nucellar	+++	++	3.0
Valencia orange	Old budline			16.8
	Nucellar	++++	+++	0

Source: After Frost (1952).

* Data from six budlings of each selection; nucellar selections originated from seed in 1915–17.

† Grades by which nucellar or low-budwood trees ranked slightly (+) to very much (++++) above the contrasted type.

Other workers, in studies on flowering and fruiting in young nucellar selections, have emphasized the thorniness of such material. Furr, Cooper, and Reece (1947) observed heavy thorns on shoots of two-year-old Jaffa orange seedlings that had developed from buds inserted into branches on older Valencia trees; the Valencia branches themselves remained low in thorns. Cooper *et al.* (1958) in Texas, reported that four-year-old budded trees from young nucellar selections of Webb Redblush grapefruit were more thorny than trees budded at the same time from the parent budline. Bitters, Batchelor, and Foote (1956) described plantings of several Valencia selections involving old budlines and recent nucellar seedling sources. Again, thorniness seemed inversely related to age of the selection from seed; recent selections such as the Campbell and Cutter Valen-

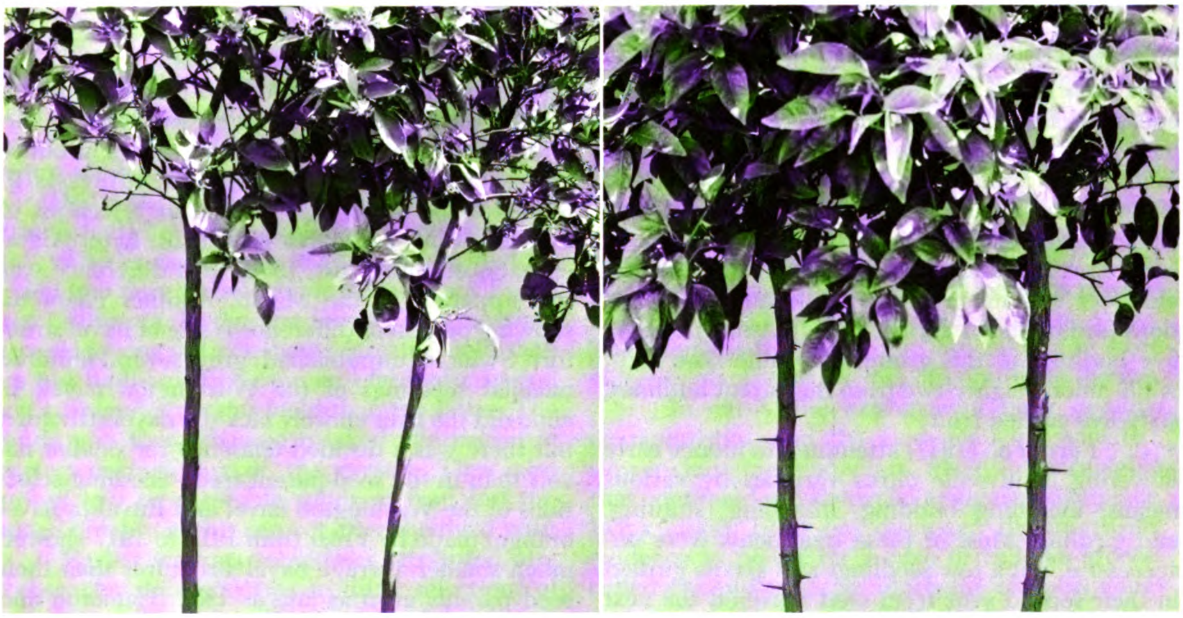

Fig. 5–4. Two-year-old Minneola tangelo nursery trees on Troyer citrange rootstock. Left, two from original budline; right, two from nucellar budline originated in 1951. Nucellar budline has more thorns, larger trunks, and fewer flowers.

cias showed rather high thorniness.

Valencia and Washington navel oranges propagated by stem cuttings from old clonal sources by Halma (1931) gave trees which for several years were more thorny than budded trees, but less thorny than seedlings. Frost (unpublished) propagated sprouts from roots of some of these stem-cutting trees; the resulting root-sprout trees were initially as thorny as seedlings, but thorniness declined rapidly. Two of these Valencia trees (one stem cutting and one root sprout) were planted next to a budded tree of Frost nucellar Valencia in 1933. By 1963, none of these thirty-year-old trees showed appreciable thorniness on peripheral branches, but recent, low, interior sprouts on the nucellar selection were thorny, while such sprouts on the other two trees were nearly thornless. Budded repropagations made from these trees in 1958 again showed an initial higher thorniness in the nucellar budline.

Growth Rate, Flowering, and Fruiting.—Important differences in growth rate and tendency to bloom, between old clonal and young nucellar selections, were early emphasized by various workers (Swingle, 1927, 1932; Tanaka, 1927*a*; Hodgson and Cameron, 1935, 1938; Frost, 1938). Swingle suggested that vigorous growth, like thorniness, may be a persistent effect of special conditions occurring during the development of the embryo. In the study by Frost (1952), the average cross-section area of trunk three years after budding was clearly greater in the nucellar budlings than in the propagations from the seed parents in nearly all of fifteen varieties. After eighteen years, many of these same nucellar trees, then in an orchard planting, showed excesses of 33 to 108 per cent in trunk area compared to the parent clones (Cameron and Soost, 1952). Tendency to early flowering and fruiting among the three-year-old trees was much lower in the nucellar selections (see table 5–1 for examples). Among eighty-five budlings of this group, only nine bore fruit, while among eighty-two from the parent budlines, sixty-five carried fruit.

Furr *et al.* (1947) attempted to induce early flowering in juvenile citrus varieties by various means, including budding, inarching, stunting, and girdling. Most of these treatments were unsuccessful, but a few seedlings and shoots girdled in their second year from seed flowered the next year. Many seedlings girdled in their sixth year flowered in the next season.

Furr (1961) compared time of first flowering and fruiting in pairs of budlings propagated from basal and top shoots of seedlings which had already fruited. Among thirty pairs of trees of Rangpur lime and Iran lemon, eleven trees from basal buds and nineteen from top buds flowered two years after budding; in the next year, nearly all trees from both bud positions bore fruit. With grapefruit, however, which is slower to come into fruit, much more early fruiting occurred from top buds than from basal ones. Similar behavior with respect to early fruiting of nucellar Lisbon lemon budlings was reported by Cameron, Soost, and Frost (1959).

Other tendencies characteristic of young seedling selections are marked biennial alternation of flower and fruit production and irregular distribution of the fruit on the tree. These differences seem to be mainly due to more extensive inhibition of flowering and fruit setting by preceding fruit production and perhaps by effects of wind and shade on the foliage.

Fruit Characters and Yield.—Fruits of early propagations of seedling selections have a tendency to elongated shape, puffing, hollowness of axis, and low seed number (Frost, 1938; Hodgson and Cameron, 1938). These characteristics are usually modified in older trees, especially after repeated repropagation. Batchelor and Cameron (1949) and Cameron and Soost (1952) found that fruit size and shape, and percentages of juice, soluble solids, and acid were generally the same in eighteen- to twenty-year-old budded trees of nucellar selections as in the comparable seed-parent budlines. Some fruit characters, however, still showed differences. Among seven varieties which produce appreciable numbers of seeds, seediness still tended to be lower in the nucellar selections of five, including the Marsh grapefruit. Later (Cameron, Soost, and Frost, 1959), this difference was still apparent in the Marsh. Another character showing persistent difference is size of navel structure. Swingle (unpublished) grew several hundred nucellar seedlings of the Washington navel; in none did the fruit entirely lack the navel structure, but there was a decided tendency for smaller navels than in the seed-parent trees. Seedling selections of the Washington navel and Ruvel (a navel orange) made by Frost from 1915 to 1917 showed much smaller average navel structures than their seed parents even as late as 1951 (Cameron and Soost, 1952). Smaller navel aperture has also been reported by Oberholzer and Hofmeyr (1955) in a nucellar Washington navel in South Africa.

In the Washington navel and the Marsh, certain undesirable differences in physical characters of the fruit have tended to persist in nucellar selections (Soost and Cameron, 1961*b*.) With the Washington, an irregular tendency to tapered stem ends and early looseness of core has existed, particularly in seasons conducive to large, slightly rough fruit. Similar tendencies, as well as a frequently thicker peel, have been observed in the grapefruit. With such characters, it is difficult to differentiate between juvenile effects and possible slight genetic changes. Somatic mutations are frequent in citrus (see p. 342). Since most of them are unfavorable, they must constantly be guarded against.

The most useful horticultural characteristics of most nucellar selections are high tree vigor and high fruit yield. Higher yields were shown in nucellar selections, as contrasted to parental budlines, in nine varieties out of ten in a study of Cameron and Soost (1952). In selections much younger from seed, however, yields are sometimes poorer than in trees from old budlines, which is due partly to the tendency to alternate bearing.

Gardner and Reece (1960) reported on characteristics of twenty-eight navel orange selections, budded on sour orange rootstock in the same orchard. These included nine Washington navels derived from nucellar seedlings in 1909 and 1910. The budded trees were rated for yield at 11 through 14 years from planting. Eight out of nine nucellar selections appear to have been among the better yielding group, whereas only nine out of nineteen of the other sources were high in yield. Most of the nucellars also ranked within the top one-half of the group in tree size.

Marloth, Basson, and Bredell (1964, and unpublished) reported preliminary results of a study of Valencia oranges in South Africa that included two nucellar selections (Olinda and Frost) from California. In the first few years of bearing, these selections did not show a greater yield than two old budlines, but over an eight-year period they averaged a greater yield than the latter. The Olinda and Frost showed marked alternation in bearing during part of the period. They also showed low total soluble solids at first, but improved notably in this respect after the third year.

Earlier workers sometimes attributed other character differences to seedling selections of normal nucellar origin. Greater frost hardiness, larger fruit size, and added resistance to disease have been reported (Traub and Robinson, 1937), but these can often be indirect or temporary effects, dependent on greater seedling vigor. Cooper, Olson, and Shull (1959) found that yellow-vein chlorosis, associated with a high water table, did not occur on virus-free nucellar selections, but was severe on old budlines affected by viruses. The difference appeared to be due to the weak root systems of the virus-affected trees.

The Question of Clonal Senescence

It is uncertain whether any of the more persistent differences between nucellar citrus selections and their seed parents reflect a physiological aging in the parent clones beyond that associated with sexual maturity. Various differences may be secondary effects of continued nucellar vigor of growth. Virus infections not showing acute symptoms can depress growth (for example, see Calavan and Weathers, 1959); injurious effects associated with the stubborn disease complex have been particularly variable. In both plants and animals, tissue-culture studies suggest that somatic cells can reproduce and thrive indefinitely if the environment is favorable enough. As mentioned earlier, certain plant clones in nature appear to be of extreme age. Cottam (1954) described a form of aspen which seems to have spread only by asexual means for perhaps 8,000 years. Hall (1929) described an ancient clone of tulip, a sterile polyploid, which was known in Italy in 1606 and still exists.

Plant forms which can maintain clonal life over very long periods usually do so by resprouting from crown, root, or underground stem tissue. This is true of such plants as olive, banana, date palm, sugarcane, and chrysanthemum. Trippi and Montaldi (1960) have postulated that the degeneration which often occurs in sugarcane clones as they grow older arises partly from the practice of propagating from the upper, and perhaps physiologically more specialized, portions of the stalks. They suggest that basal cuttings may give more vigorous plants that are less susceptible to disease.

In citrus, the existence of clear juvenile characteristics, and the evidence for associated, slower-changing characters, make it conceivable that continued physiological aging can take place. Brink (1960, 1962) has discussed the increasing evidence in plants for a type of cellular process called paramutation by which the classical genes may sometimes interact with other chromosomal components during somatic development, so that somatically permanent changes occur both in the chromosomal component and in the cellular end products of the gene action. In some cases, chromosomal components are so modified that the change persists in subsequent sexual generations. Brink postulated that phase change, including sexual maturity, in plants may be dependent on a normal form of this mechanism. If this is so, additional changes might also be brought about by such causes during the life of a clone.

Nucellar Embryony and Citrus Variety Improvement

The information derived from long-term studies of the effects of nucellar embryony has had an important impact upon commercial citrus production in the United States and elsewhere. The investigations described on the preceding pages, and the extensive information developed by plant pathologists on the behavior of citrus viruses, have led to widespread use of selected nucellar budlines for the establishment of vigorous, relatively virus-free citrus orchards. Since most citrus viruses are seldom seed-borne, nucellar-seedling budlines have made it possible to establish desirable rootstock-scion combinations which would often fail if older, virus-infected bud sources were used. The superior health and vigor of these combinations commonly result in longer-lived, higher-yielding trees, even though some problems associated with juvenile characteristics still remain in a few varieties.

In California, the majority of all orange and lemon trees now (1968) propagated by nurserymen, and a substantial proportion of the grapefruits and mandarins, are derived from budlines of known nucellar origin. In many other citrus-growing areas of the world, including South Africa, Central and South America, and various Mediterranean countries, the commercial use of such budlines is rapidly increasing, both by the introduction of California selections and through the development of local sources.

The pioneering research of W. T. Swingle, H. B. Frost, H. S. Fawcett, R. W. Hodgson, and L. D. Batchelor, together with studies by later workers representing the combined disciplines of horticulture, plant breeding, and plant pathology, can be largely credited with making this development possible.

GENETIC VARIATION

General Principles

Genetic variations originate through several processes that are fundamentally distinct. Most genetic variations depend on new combinations of genes, which are carried on the chromosomes. New combinations in the offspring are either the direct result of hybridization or arise from segregation and recombination of genes within a hybrid parent. Recombinations are usually produced abundantly in sexual reproduction, which is the main reason seedling fruit trees differ genetically from their parents. Occasionally, change occurs in the nature of the gene; this has been called "point" mutation. Changes resembling point mutations in their effects on the organism can be produced by chromosome aberrations, or by abnormal segregation of chromosomes, leading to loss, duplication, or rearrangement of genes. *Somatic* segregation signifies gene segregation in somatic cells; this may occur in an occasional cell by unusual crossing-over, abnormal chromosome disjunction, or other exceptional processes. Finally, tissues of two different genetic types may become combined in the same plant, either side by side or one within the other, forming a chimera.

The processes listed above may be grouped into three categories: (1) the regrouping, duplication, or loss of unchanged genes within cells; (2) change in the nature of the gene; (3) the combination or rearrangement of cells of two or more genetic types in one individual (chimeras).

If a genetic variation first occurs in the germ cells, it is a gametic or germinal variation; if it arises in the soma or body of a plant, it is a somatic or bud variation.

A large body of evidence shows that heredity, in higher organisms at least, depends primarily upon the chromosomes. Chromosomes have also been identified in certain fungi and bacteria, where they had formerly been undetected. However, certain forms of extra-chromosomal inheritance are likewise well established. Transmission through the cytoplasm of abnormal plastid characteristics, such as albinism, is well known. Numerous cases of cytoplasmically controlled male sterility are also on record. Evidence for phenomena such as paramutation (see p. 331) is more complex and not yet fully understood.

Genes and Chromosomes

The concept of genes as macromolecules, or combinations of such molecules, arranged in linear order on the chromosomes, remains important to genetics. In somatic cells of diploid higher plants, the chromosomes are present in duplicate; the two chromosomes of a pair, which are similar in their gene content, are homologous chromosomes. When a somatic cell divides, each chromosome separates longitudinally, and each of the two daughter cells normally receives chromosomes and genes identical with those of the mother cell. In two cell divisions closely preceding the formation of the gametes, in contrast, the two homologous chromosomes pair and then separate so that each gamete receives only one chromosome from each pair, and the total number of chromosomes is reduced to one half. These two reduction divisions constitute meiosis. When an egg and a sperm unite, the full somatic number of chromosomes is restored; the embryo thus usually receives half of its genes from its mother and half from its father.

The process of chromosome duplication—including gene duplication—has become much better understood in recent years. Precisely arranged linear sequences of chemical units are assembled within the cell to reproduce exactly the already-existing chromosome strands, apparently with the latter serving as templates. Sequences of units, which correspond to functional locations of genes, can sometimes be delimited. Some gene effects are controlled by complex loci that behave as though they were composed of several subunits.

Genes situated at corresponding positions (loci) in homologous chromosomes are allelic. When the chromosome pairs segregate at meiosis, such genes, of course, also segregate. Genes at different loci on the same chromosome (linked genes) can normally be separated only through crossing-over, which is an exchange of parts occurring between chromosomes of the same pair during the reduction divisions. Nonhomologous chromosomes, together with the genes they carry, recombine freely during the reduction division. New genetic combinations can occur in progeny by such regrouping of genes in either or both parental gametes.

If two allelic genes are identical, the individual is homozygous for this gene pair. Each gamete then receives one of these two genes; all the gametes, and all offspring from selfing, are thus alike with respect to this pair of genes. If a plant is homozygous for all its pairs of genes, it normally produces only one kind of gamete. Upon selfing, its offspring will all be genetically identical, that is, it will "breed true." If allelic genes are not identical, the individual is heterozygous for this gene pair; half of the gametes will receive one kind of gene and the other half will receive the other kind. Such a parent will not breed true; its offspring from selfing will differ in any characters that depend on the differences between these two genes.

The genes of an organism jointly direct its life processes and, within limits, determine its characteristics. Genes can best be identified and studied when one or a few gene differences produce clear differences in the organism.

Hybridization, Hybrid Vigor, and Inbreeding

The word hybrid as used here refers to forms which are heterozygous as a result of interbreeding of unlike parents, regardless of remote ancestry. Probably all citrus forms are somewhat heterozygous, because of ancestral mutation and crossbreeding. In many kinds of plants, different species within a genus will not intercross or if they do they may produce sterile hybrids. In *Citrus,* however, interfertility of species is usual, and considerable fertility of the F_1 hybrids is common.

The genera *Poncirus* and *Fortunella* (the kumquat) can be crossed with *Citrus* and some of the hybrids are somewhat fertile. But Swingle and Robinson (1923) found it difficult to cross *Poncirus* with *Fortunella,* and nearly all the hybrids were weak. Citranges have been crossed with *Fortunella,* giving some vigorous hybrids. Toxopeus (1936) obtained weak hybrids from the cross of *Citrus aurantifolia* (Christm.) Swing. with *Murraya paniculata* (L.) Jack. Traub and Robinson (1937) described a hybrid between *Eremocitrus* and a citrange. Efforts at Riverside to cross *Severinia* with *Citrus* have been unsuccessful.

High variability of zygotic progenies seems characteristic of at least all citrus forms which produce nucellar embryos. Variability occurs whether the parents belong to the same or different species or to distinct genera. Some of the first citranges produced by controlled hybridization differed among themselves more widely than do various varieties within the parent species (Swingle, 1913). Similar variability has been generally observed by citrus breeders. Most characteristics are affected, including size, shape, and vigor of tree, susceptibility to disease, and many fruit characters. Although a variably intermediate condition is the most common, certain characters may closely approximate those of one parent, or even be outside the parental range.

There is evidence for hybrid vigor and inbreeding depression in citrus, although critical proof is lacking. Webber and Swingle (1905) reported citranges to be more vigorous than nucellar progeny of the parent species; citrange crossed with grapefruit also gave high vigor, as did kumquat with citrange. The authors have obtained small populations of *C. grandis* (L.) Osbeck × *P. trifoliata* (L.) Raf. (trifoliate orange) and of a zygotic hybrid (*C. sinensis* [L.] Osbeck × *C. paradisi* Macf.) × trifoliate orange, which are highly vigorous. Evaluations of vigor in young seedlings must take into account the effects of competition from nucellar embryos when these are present.

Within *Citrus*, it appears that wider crosses are the more likely to produce vigorous offspring, but exceptions are not uncommon. Interspecific crosses involving *C. grandis*, like intergeneric ones, appear to be unusually fast-growing. Frost (1943), in limited tests, reported weak hybrids from the narrow crosses of Ruby × Valencia orange and Eureka × Lisbon lemon, and Torres (1936) found that even the wider cross, sour orange × rough lemon, gave hybrids which were weaker than either parent. Within the diverse mandarin group, various crosses have given considerable numbers of vigorous hybrids, yet at the University of California Citrus Research Center, Riverside, present studies suggest that backcrosses and "near" backcrosses among mandarin types are not as vigorous as second-generation hybrids involving two or three *Citrus* species. New hybrids of Eureka lemon with Lisbon lemon (R. K. Soost, unpublished) show poor average vigor.

Clear evidence on the effects of selfing is difficult to obtain, since many polyembryonic varieties give few zygotic seedlings. Self-incompatibility, present in some citrus forms (see chap. 4) further interferes with selfing studies. Weakness of variants from selfing or presumed selfing (fig. 5–5) was reported by Webber and Barrett (1931) Webber (1932) Toxopeus (1936) Torres (1936) and Frost (1943). At Riverside, California, recent orchard plantings of sexual progeny from selfing of a hybrid of *C. grandis* (L.) Osbeck × *C. reticulata* Blanco are not as vigorous as F_1 or advanced-cross

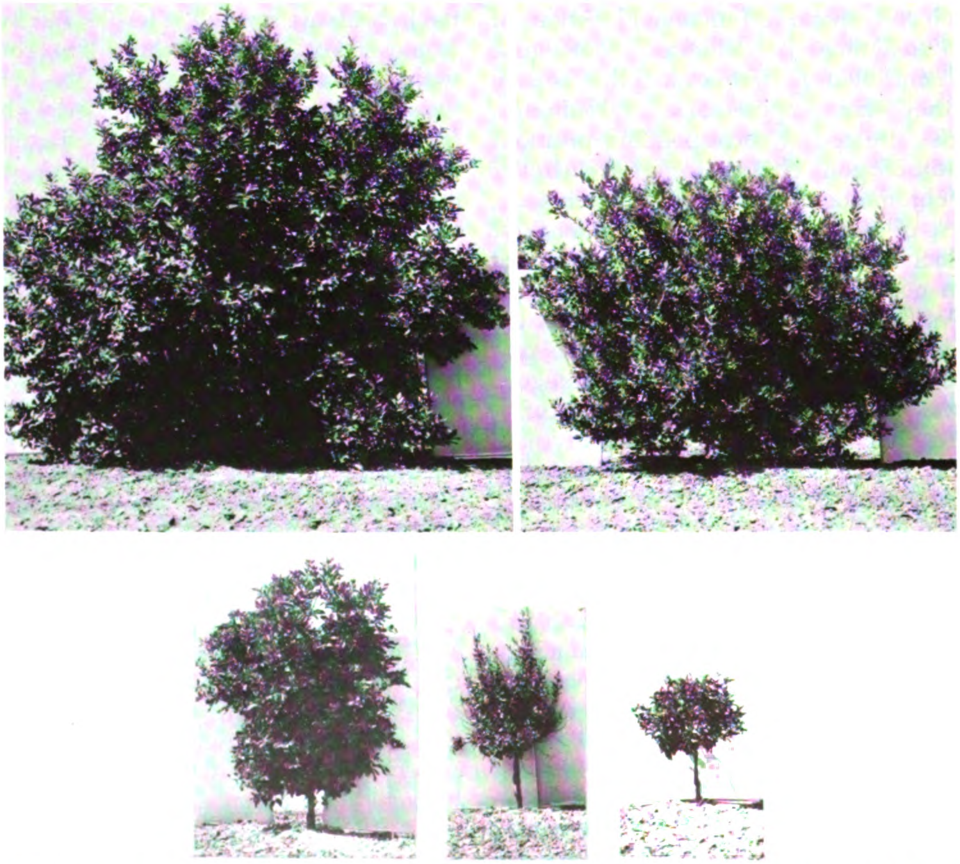

Fig. 5–5. Weakness of selfed progeny from Lisbon lemon. Upper left, nucellar Lisbon; others, sexual progeny. All are budded trees, five years old in the orchard, photographed from the same distance.

trees involving comparable parents. J. R. Furr (unpublished) has obtained populations from selfing of the zygotic Temple, and many of the plants seem initially vigorous. Temple (tangor?) is apparently a hybrid, and perhaps its selfs (and those just cited from Riverside) are not as weak as selfs within a homogeneous and polyembryonic group such as the sweet oranges.

Nucellar Embryony and Heterozygosis in Citrus Evolution

Nucellar embryony must have had an important relationship to natural and artificial selection in the evolution of *Citrus*. Natural selection no doubt acted not only on trees, but on embryos during seed formation. To be successful under natural selection, citrus forms had to possess adequate growth vigor and climatic adaptation and also satisfactory capability of seed reproduction.

Abundant heterozygosis is present in most citrus forms; the agencies responsible for this are presumably: (1) frequent gene mutation; (2) cross-pollination within a wide range of interfertility; and (3) nucellar embryony. Reproduction by nucellar embryony preserves any heterozygosis that may originate by hybridization or mutation. It thus must have favored accumulation of mutant recessive genes, including those detrimental to vigor and fertility. When such genes became numerous, they would have tended to eliminate successful sexual reproduction, especially by selfing. Hybrids between forms not closely related would have had better prospects of survival, because of greater freedom from homozygosis. Nucellar embryony, by providing for asexual reproduction, acts as an isolating agent which should favor evolutionary differentiation.

Even without asexual reproduction, mutation and frequent cross-pollination should tend toward the accumulation of unfavorable recessive genes. If nucellar embryony should then originate, forms with this character would probably be preserved by natural selection. But excessive evolutionary increase in the number of embryos per seed should be lessened by the unfavorable effects of reduction of embryo size.

Many forms of *Citrus,* and their progenitors, may have been reproduced almost entirely through nucellar embryos from remote antiquity. Differentiation of varieties, in such a group as the sweet oranges, may have occurred mainly by somatic change without frequent sexual reproduction. Occasionally an exceptional recombination of genes from heterozygous parents might have given rise to a different and horticulturally valuable new type. Such a type might be so unlike the parents that its origin would remain uncertain.

Tanaka (1927*a*) concluded that such abrupt origin of radically different types has occurred, but he considered that mutation, rather than recombination, probably was the main cause. He suggested that the satsuma, the Mediterranean mandarin, and the grapefruit may have originated in this way, and he indicated forms which seem closely related to these. For the grapefruit, the obvious relative is the pummelo, which lacks nucellar embryony and seems to be much less heterozygous than most citrus forms. Origin by interspecific hybridization has also been suggested for the grapefruit; if this occurred, segregation and recombination evidently established a definite new type. Traub and Robinson (1937) suggested that the grapefruit seems intermediate between sour pummelos and "salad" pummelos.

The low variability among the true lemons, and among the sweet oranges, may represent mainly somatic variation. Mandarins, however, have a broader genetic base, and suggest more differentiation by sexual reproduction.

Comparatively wide crossing in *Citrus* has no doubt been more common under domestication than in earlier times, because forms have been brought together under cultivation. Although Tanaka (1927*a*) considered that definite hybrids among the varieties of the Pacific region were rather few, he (1961) described several probable cases, including the Benikoji, the Funadoko, and the Iyo. Many Indian citrus varieties described by Bonavia (1888–90) had characters suggestive of hybridity between lemon and citron. Luss (1935) concluded that the Ponderosa and Melarosa lemons and the Asahikan "pomelo" are interspecific hybrids. Other probable natural hybrids include the Rangpur lime, Meyer lemon, Japanese citron, and Temple orange (tangor?).

Inherited Characters in Citrus

Citrus normally has nine pairs of chromosomes, which carry perhaps thousands of genes. For a number of reasons, little has been proven as to the effects of the individual genes. Each tree occupies considerable space and usually requires several years from seed to fruiting. The frequent presence of nucellar progeny, which may not be identifiable until fruiting, complicates genetic studies. Zygotic seedlings from any one cross are highly variable, and those from close inbreeding are often weak or sterile, making gene analysis difficult.

Occasionally a conspicuous character difference and a fairly simple progeny ratio suggest segregation for one pair of genes. Thus, when the dwarf and short-petioled Bouquet sour orange was crossed with the Imperial grapefruit, about half the hybrids had very short petioles (fig. 5–6); nearly all of these trees also resembled the Bouquet in other foliage aspects (Frost, 1943). But when the Maltese Oval orange was crossed with the Bouquet, the hybrids were not sharply separable by these characters. Perhaps one chromosome of the Bouquet carries a gene with major effect on leaf form, while the allel on the homologous chromosome has less effect. Such an effect was obviously modified when Maltese Oval was the other parent.

Toxopeus (1936) reported segregations for dwarf plants from the cross of a pummelo *(Citrus grandis)* with a citron (*C. medica* L.). When the pummelo was used as seed parent, only dwarf hybrids were obtained; the reciprocal cross produced 50 per cent dwarfs. Such ratios can occur when one parent is heterozygous for a dwarfing gene and there is also elimination of the allelic normal gene from the gametes of one sex but not the other. At the University of California Citrus Research Center, several progenies involving a particular pummelo, obtained in 1945 and 1946, have also shown segregations for extreme dwarfs. Hybrids of the Karn Lau Yau pummelo (CRC 2341) as female, crossed by Clementine mandarin, were exclusively dwarfs, which remained only about 20 to 30 cm high at three years of age. Crosses of this pummelo with Temple, Kara, and Valencia each produced some but not all dwarfs. Normal siblings were many times larger than the

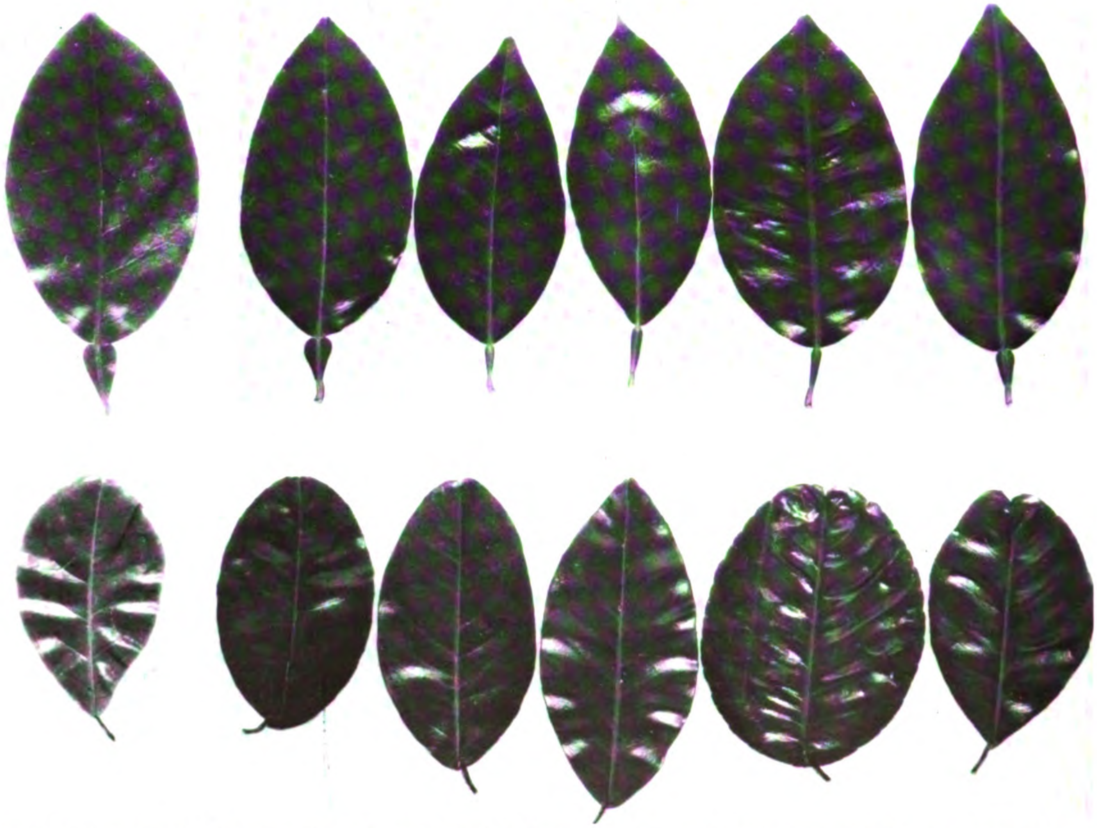

Fig. 5–6. Leaves of the parents and ten hybrids from the cross, Imperial grapefruit × Bouquet sour orange. Leaf at far left in upper row, Imperial; leaf at far left in lower row, Bouquet. The five remaining leaves in the upper row are from hybrids with petiole lengths resembling Imperial; the five remaining leaves in the lower row have very short petioles similar to Bouquet.

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dwarfs. When plants which died in their first year, without making appreciable growth, were included as probable dwarfs, the proportion of dwarfs ranged from 42 to 62 per cent in these families. In contrast, a progeny of 176 plants of the same pummelo crossed by the Pearl tangelo showed no dwarfs. A different pummelo, Moanalua (CRC 448) when crossed by an orange, yielded a lower proportion of dwarfs. A single major gene could be involved. In the Riverside progenies, after several years, one dwarf and a budling, probably from a different dwarf, began to grow vigorously, indicating a radical change in physiology. Fig. 5–7 shows two dwarfs and one normal plant.

There is evidence that within the genus *Citrus*, nucellar embryony is inherited in a rather simple fashion. Parlevliet and Cameron (1959) found that hybrids from sexual, monoembryonic parents were themselves always essentially monoembryonic. Crosses of monoembryonic by polyembryonic parents of several *Citrus* varieties yielded hybrids of both types in ratios which often approached 1:1; certain parents conditioned higher proportions of polyembryonic offspring. Monoembryonic offspring were occasionally obtained from polyembryonic parents. Nucellar embryony may be principally controlled by one dominant gene; monoembryonic varieties would then be homozygous for the recessive allele.

Recent crosses of the Clementine mandarin by *Poncirus trifoliata* (monoembryonic × polyembryonic) have given different results in F_1. Thirty-one out of thirty-two hybrids tested appear to be monoembryonic.

Evidence that certain essentially monoembryonic varieties do not produce nucellar embryos at all was obtained by Ozsan and Cameron (1963). (See Chapter 4, p. 302.)

Levels of acidity in citrus fruits are also inherited. Both acid and essentially acidless forms are known in many varieties. Soost and Cameron (1961*a*) obtained hybrids of relatively low acidity from crosses of a low-acid pummelo by medium-acid pollen parents of several varieties. Other pummelos of rather high acidity, when crossed with the same pollen parents, produced progeny whose acid levels were mostly above those of either parent. The gene basis is not known; it was noted that the differences in acidity were not closely correlated with differences in levels of soluble solids.

The trifoliate leaf character of *Poncirus* is known to show dominance in crosses. In the F_1, it is nearly always fully dominant over the monofoliate leaf of *Citrus;* a few hybrids occur which show mixtures of bifoliate and monofoliate leaves. Patterns of segregation in advanced generations do not indicate single gene inheritance. Some unifoliate seedlings from open pollination of F_1 plants are produced, but the proportions are low. Yarnell (1942) grew open-pollinated progenies from citranges and citrumelos. A total of fifty seedlings from six zygotic citrumelos included nine unifoliate and seven variably unifoliate plants. Among 3,589 seedlings from twelve highly nucellar citranges, segregants of any kind were rare as would be expected. Webber (1932) had earlier reported leaf segregation in open-pollinated progenies of only one (the Sanford) of six citrange varieties studied.

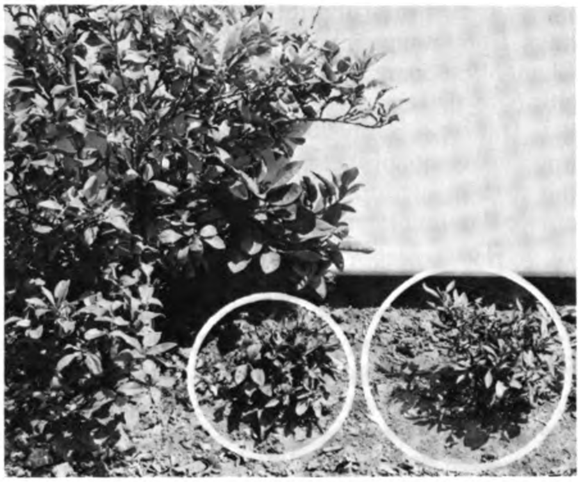

Fig. 5–7. Dwarf and normal nursery plants from the cross, *C. grandis* × *C. reticulata* 'Clementine.' Dwarfs (in circles) are about 10 inches in height.

The authors have studied leaf segregation in open-pollinated progenies from a series of highly zygotic F_1 hybrids of the parentages Clementine × trifoliate orange, and Sukega grapefruit × trifoliate orange. Second generation seed was obtained where location and flowering times almost insured that only selfing or crossing among the hybrids had occurred. From a total of 1,716 seedlings from thirty-three hybrids with Clementine, 7.17 per cent were monofoliate. From 326 seedlings from seven hybrids with Sukega, 5.8 per cent were monofoliate. A small added percentage of each group were fluctuatingly bi- or trifoliate. Classification as monofoliate was based upon seedlings which had reached at least the eight-leaf stage.

J. R. Furr (unpublished) has grown seedlings from monoembryonic Temple tangor and Clementine mandarin pollinated by the Troyer citrange; these represent backcrosses to monofoliate leaf types. Only 20 to 30 per cent of the seedlings were monofoliate. These data appear to fit the pattern of duplicate factors by which either of two dominant genes conditions trifoliate leaves.

Toxopeus (1962) reported that among 50 two-year-old seedlings from the cross of *C. grandis* (L.) Osbeck × *C. hystrix* DC. (both monofoliate) about one third were trifoliate-leaved. He suggested that two complementary, dominant genes may be involved, with each species being heterozygous for one of them. This gene system does not account for the breeding behavior of *Poncirus,* just described. Toxopeus also found that in the cross of *C. grandis* (three varieties) × Meyer lemon about 50 per cent of 598 seedlings showed in young leaves the purple color characteristic of lemons; he postulated that the Meyer may be heterozygous for a dominant gene for this color.

The trifoliate orange is notably resistant to the citrus nematode, *Tylenchulus semipenetrans* Cobb (DuCharme, 1948; Baines, Bitters, and Clarke, 1960), and it transmits this resistance to its F_1 hybrids with *Citrus,* as measured by root infestations of young seedlings in the greenhouse. Cameron, Baines, and Clarke (1954) found that 95 per cent of 484 hybrid seedlings, involving five *Citrus* species, showed low infestation, as did 100 per cent of 846 *Poncirus* seedlings. From 63 to 100 per cent of the nucellar seedlings of the *Citrus* parents became heavily infested. Older trees of *Poncirus* hybrids in the field do not show such consistent resistance.

In Florida, search for resistance to the burrowing nematode, *Radopholus similis* (Cobb) Throne has lately been the subject of intensive study. Great differences in susceptibility have been reported among *Citrus* varieties, selections, and relatives (e.g., Ford and Feder, 1961). Among more than 1,200 forms tested by 1962, nearly all have been found susceptible, although a few specific source trees within several taxonomic groups are tolerant or resistant. Two rough lemon selections, six sweet oranges, and the Carrizo citrange are classed as tolerant. Ridge pineapple sweet orange and Milam, a lemon hybrid, are resistant. Among twelve nonsusceptible selections, seven are sweet orange types, but these fall among several subgroups. Such isolated instances of resistance suggest unexpectedly specific genetic differences, especially since some of the selections are in most characters highly similar to susceptible members of their groups.

A striking range of segregation for tree and fruit characters was described by Reece and Childs (1962) among about seventy seedlings from seed collected from pulp of the nearly seedless Persian lime. It was concluded that the Persian lime can segregate for many interspecific characteristics, presumably after selfing. The Persian lime studied by Bacchi (1940) was found to be triploid, as was the Bearss lime (Krug and Bacchi, 1943); Nakamura (1934), however, reported diploidy in the Persian lime. A wide range of characters is not unlikely in the progeny of a triploid, especially if it is a hybrid. However, some of the seedlings in question probably arose by outcrossing or other contamination. Two seedlings were found to be essentially identical to the Persian lime (Childs and Long, 1960) and thus seemingly of nucellar origin. If they are not nucellar, they would represent unusually rare products of sexual reproduction.

Data on inheritance of salt tolerance, *Phytophthora* tolerance, and cold hardiness are discussed on pages 362–63. Segregation for male sterility is described on page 366.

Mutation

In a broad sense, mutation may be defined as change in genetic constitution which arises suddenly and is not due to segregation and recombination of genes in sexual reproduction. Mutation may occur either in germ cells or somatic cells. More narrowly, gene mutation signifies change of a gene to a somewhat different gene. As noted on page 332, loss or rearrangement of chromosomes or their parts or inherited changes in cytoplasmic particles may simulate gene mutation in their phenotypic effects. Heritable bud variations and chimeral conditions usually are the results of mutation in one form or another.

Even in the best-analyzed organisms, it is often impossible to distinguish between gene mutation and the loss of a minute part of a chromosome which included that gene. However, there is evidence in lower organisms for reversion, or back mutation, of genes. In such cases, the gene apparently could not have been lost. In other cases, the action of a gene can be shown to have been suppressed by another gene.

Among sexual seedlings of citrus, it is rarely possible to distinguish new gene mutations from

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variations due to normal recombinations of genes from the parents. Among variant nucellar seedlings, or variant branches or fruits, gene mutation is likewise seldom separable from grosser chromosome changes. The possibility of chimeral conditions introduces uncertainty, since tissue of one genetic type may remain within that of another for an indefinite time, and then emerge to produce a new effect.

Unstable Genetic Factors in Citrus.—In some sectorial fruit-rind chimeras, the visible changes appear to be caused by unequal nuclear division rather than by gene mutation. A fruit may have two adjoining variant sectors of rind, often about equal in width, which are changed from the normal in opposite directions (for an early example, see Toxopeus, 1933). Thus, one sector may be abnormally thick and a contiguous sector abnormally thin. Since one member of such a pair of variations may often be inconspicuous, or entirely lost (perhaps due to growth competition), rind chimeras showing single sectors may often also be the result of unequal nuclear division. Various bud-variation types often differ from the parent type in several characters, which leaves it doubtful whether the original change was limited to one gene. In maize, Jones (1937) concluded that certain endosperm changes were produced very much oftener by somatic segregation than by gene mutation.

When mutations occur in citrus, their expression in somatic tissues should be favored by the apparently extensive heterozygosis of most forms. Such heterozygosis, in turn, implies that mutation has occurred frequently in the past. Certain unstable types, such as are discussed below, could result from gene mutation.

In an early study at Riverside, California, six of the nucellar offspring of one Valencia orange tree bore fruits that were partly without

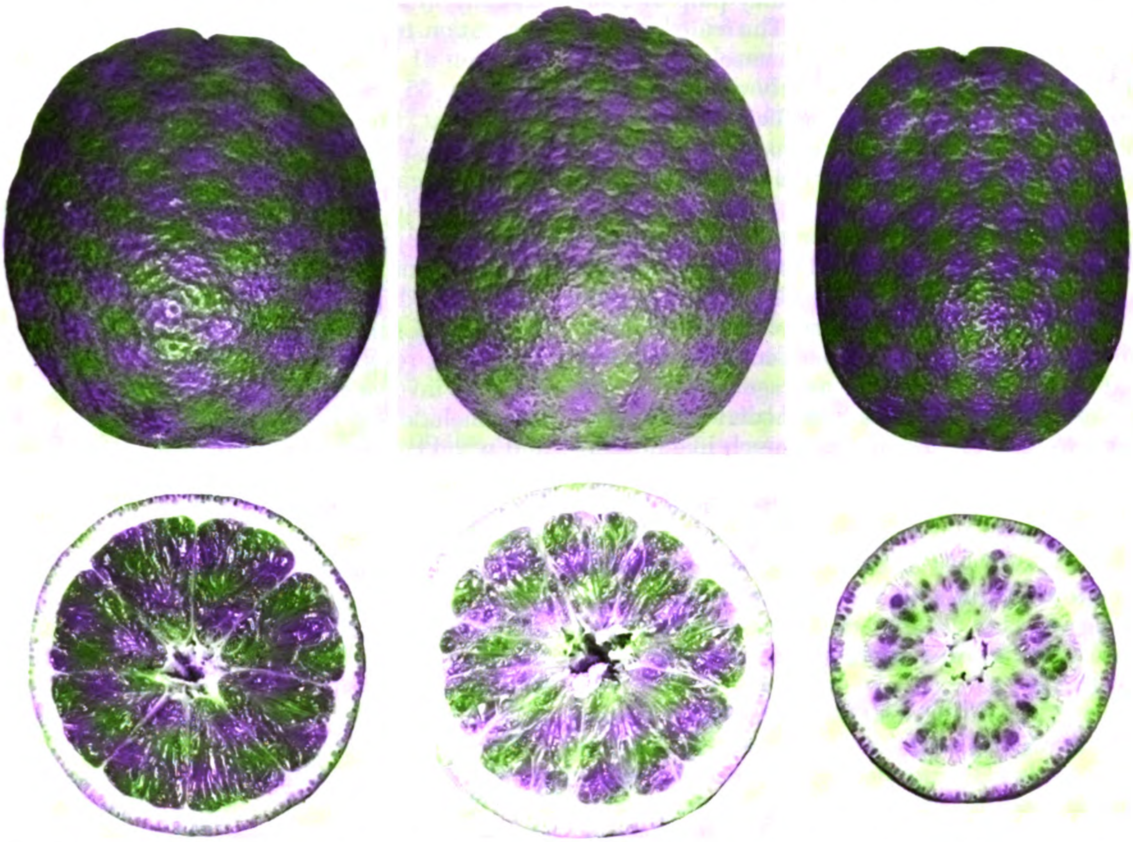

Fig. 5–8. Fruits from three nucellar seedlings of Valencia orange. Left column, normal fruit from a seedling of a normal parent. Middle column, thick-rinded and very firm fruit from a seedling of a parent with occasional abnormal rind sectors; right column, small dry fruit from another seedling of the same parent. About 2/3 natural size.

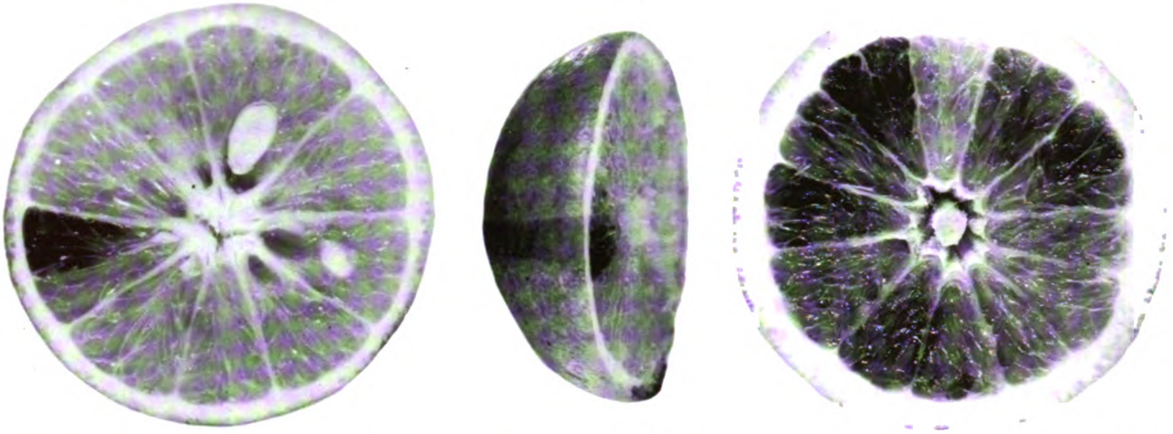

Fig. 5–9. Ruby orange fruits with variant color sectors. Left and center, two views of a fruit having half of a pulp segment and overlying outer rind with increased red color. Right, a fruit having a segment at top with much less red than adjoining segments.

juice (Frost, 1943). The fruits varied from abnormal firmness and toughness of juicy pulp to complete absence of juice (fig. 5–8). The fruits on each tree differed greatly among themselves, but in addition the affected trees clearly differed in average amount of fruit dryness. This behavior can still be observed in trees more than thirty years of age in the orchard, which are the budded progeny of the original seedlings.

A pollenless navel orange of unknown origin, known as Ruvel, produced in five separate nucellar seedlings from two small branches a pollen-bearing and mainly non-navel form called Rufert (Frost, 1943). Trees budded from different seedlings have differed in average grade of the new characters; some are intermediate, and some vary within the individual tree much like the Valencia progeny described above. Their characteristics, although variable, have been maintained by budding. The Trovita orange (Frost, 1935) is somewhat similarly variable. Three seedlings from a single fruit gave rise to the Trovita type; trees propagated from one of these has produced frequent navel-marked fruits and fewer seeds than the others.

The red flesh color and rind color of the Ruby orange may be genetically unstable. Variant rind sectors with increased or decreased redness occur frequently, and segments and half-segments showing a discontinuous increase or decrease in pulp redness have been observed (fig. 5–9). In addition, nucellar progeny trees from seeds of a single parent tree at Riverside have included more than one genetic color type. Trees budded from different seedlings have differed greatly and persistently in average pulp color. In a first-budded generation trial between 1929 and 1935, nucellar selection 41 had a much higher color rating than selection 35 (table 5–2). In the second-budded generation, the selections maintained this relationship and both appeared to differ from budded trees of their seed parent. The red color is very susceptible to environmental modification, but the relative intensities in these selections have been consistent for many years.

Wood pocket disease or ligno-cortosis of semidense Lisbon lemons (Calavan, 1957*a*, 1957*b*) appears to result from an unstable genetic factor and may involve chimeral interrelations. Its symptoms include leaf variegation, fruit rind sectoring, and wood cankers. A similar or identical disease, sometimes called lime blotch, is known in Tahiti limes (Gates and Soule, 1950). Wood pocket disease recurs in lemon trees grown from buds or grafts from diseased sources, and it is transmitted through a considerable proportion of both zygotic and nucellar seedlings. In contrast to citrus virus diseases, however, it is not transmitted to healthy trees by tissue grafts, and its known occurrence is limited to certain lemons and limes. Degree of symptom expression is variable, but there is a strong tendency for the disease to increase in severity in successive budded or seedling generations.

Bud Variation.—Mutations resulting in bud variation are very prominent in citrus. The mechanisms which can be involved were noted on page 332. In a plant which is a synthetic chimera, bud

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Table 5–2

LEVELS OF RED COLOR IN THE FLESH OF NUCELLAR RUBY ORANGES AND THEIR SEED PARENT IN TWO BUDDED GENERATIONS[*]

	Budded Generation of Nucellar Seedlings									
	First Generation				Second Generation[†]					
Budline	Number of Trees	1929	1930	1933	Number of Trees	1938	1939	1948	1949	Average (All Years)
Seed parent budline	..	....	....	....	2	4.9	2.7	5.0	4.4	4.3
Nucellar selection 41	1	4.1	2.6	3.3	2	3.8	1.8	3.4	2.9	3.1
Nucellar selection 35	1	0.8	0.6	1.5	2	1.6	0.7	0.7	1.4	1.0

[*] First budded trees planted in 1920, second budded trial in 1933; all on sour orange rootstock. Color ratings based on a scale of 0 (none) to 7 (most) on cut halves of twenty-fruit samples. Trees grown in Riverside, California.

[†] Budded generation not known for seed parent.

variation can occur without having been preceded by mutation.

A somatic mutation presumably may originate in any cell. If the new cell type can reproduce itself, a sector descended from it may develop, and the shoot or fruit becomes a chimera. Sooner or later, unmixed shoots of the new type may be produced.

In studies of bud variation, one must distinguish between environmental variations and occasional heritable changes. Only when a bud variation is markedly different in tree or fruit is it likely to be detected among the environmental fluctuations which are commonly present. A new form differing only slightly in some quantitative character such as yield or fruit size will often be overlooked, and would require extensive trial to demonstrate its genetic difference from the parent type.

A. D. Shamel and associates, of the U.S. Department of Agriculture, carried out a long series of studies on bud variation in citrus, which extended over the period from about 1909 to 1936 (Shamel, 1943). Shamel sought out and described several score of variations occurring among important commercial varieties, principally in growers' orchards in California and Arizona. The variations occurred both as limb sports and whole trees; the permanence of many of these changes was demonstrated by bud propagation and performance records of the normal and variant progeny types. In addition, many single-fruit chimeras were observed that could not be propagated.

Shamel classified the variations that he studied as morphological or physiological, and as physical or chemical. Variations were found which affected tree or leaf size, tree habit, chlorophyll production, fruit size or shape, rind thickness, and acidity. These classifications, of course, represent the end results of various genetic and developmental interactions.

In commercial orchards of Washington navel and Valencia oranges and Eureka lemons surveyed by Shamel beginning about 1910, an average of some 25 per cent of the trees were judged to be entire-tree variations. The percentages ranged from less than 10 per cent to more than 75 per cent, depending upon the orchard; limb variants of the same types were also often found. It seemed clear that the whole-tree variants had arisen from budwood taken unintentionally from mutant twigs or branches on budparent trees, and that the same mutations were sometimes recurring on normal progeny trees. Single-fruit variations also occurred on many trees.

From surveys of the Washington navel variety, Shamel and his associates designated at least twenty-four variant types. These included unproductive variants, some of which were classed as "Australian" types, variants with rolled or slender leaves, and many variants bearing different sorts of abnormal fruits. Among other citrus varieties, some fifteen variant types were described in the Valencia variety, about eight major types in the Eureka lemon, and six in the Lisbon lemon. Several variants were also recognized in the Marsh grapefruit and in other varieties less extensively studied. The classes of changes observed were rather similar among all varieties. Table 5–3 is a summary of data from Shamel (1943) showing the characteristics and progeny performance of some of the unfavorable bud variations and their normal counterparts.

Several bud variants showing potentially useful characters were described by Shamel. One

Table 5–3

PARENT VARIETY AND BUD-PROGENY CHARACTERS OF SOME BUD VARIANT AND NORMAL SELECTIONS IN CITRUS

Parent Tree		Budded Progeny Trees*		
Variety	Tree or Limb Type Selected	Number Tested	Fruit Type	Fruit Yield (Total Number)
Washington navel orange	Unproductive	2	Normal	544
	Normal	2	Normal	4,703
Washington navel orange	Unproductive, coarse	1	Coarse	1,180
	Normal	1	Normal	2,654
Washington navel orange	Small-fruited "Australian"	1	Small fruit	1,414
	Normal	1	Normal	3,332
Thompson navel orange	Unproductive, coarse	1	Coarse	310
	Normal	1	Normal	3,576
Valencia orange	Unproductive	2	Near normal	1,196
	Normal	2	Normal	6,328
Valencia orange	Willowleaf	1	Small, coarse	1,288
	Normal	1	Normal	5,395
				Lb Per Tree Per Year
Eureka lemon	Pear-shaped	10	Elongate, necked	114
	Shade-tree	18	Thick rind, little juice	70
	Normal	72	Normal	197
Lisbon lemon	Dense, unproductive	17	Thick rind, low acidity	48
	Normal	42	Normal	346

Source: After Shamel (1943).

* Records made over a 4- to 12-year period between 1921 and 1933.

of these, a limb variant of Washington navel designated Everbearing, flowered over a considerable period and bore dense clusters of fruit. Thorns on this variety sometimes bore flowers and fruit. Another productive variant of the Washington, called Vivial, bore fruits without navel structures. The Robertson navel (Shamel and Pomeroy, 1935) is a variant usually ripening slightly earlier than normal Washington from which it arose.

Since most bud mutations are undesirable, Shamel rightly emphasized the importance of careful selection of budwood from source trees typical of the variety and preferably with proven performance records. Much of his work consisted in helping to establish dependable sources of normal or possibly improved selections of existing citrus varieties.

Numerous bud-mutation types, in addition to those discussed above, are known or believed to have arisen from the Washington navel orange, which itself is considered to be a sport from the Selecta orange. Variants that have received some recognition include the Thomson, Carter, and recently, in California, the Gillette and Newhall. Most of these variants appear to be slightly earlier or sweeter than the Washington. Another mutant, the Marrs, has attained prominence in Texas (Waibel, 1953; Olson, 1963), where it was discovered as a limb sport in 1927. In Texas, the Marrs shows several differences from the Washington, including greater productivity, earlier marketability, smaller tree size, fertile pollen, and a lack of bitterness in the juice. The fruit seldom has a navel.

The Salustiana orange, reported to have arisen in Spain as a bud sport of the Comun, ripens early in North Africa (Chapot and Huet, 1963). It is almost seedless, juicy, and highly productive.

A recent apparent bud mutation, resulting in an inferior type, is a high-acid form of navel orange recognized in California in 1960 (Soost *et al.*, 1961). The strain was through an accident widely propagated, so that many young plantings of supposedly normal Washington navels contained varying numbers of trees with the variant characters. Growth habit of the trees was unusually upright, vigorous, and thorny. Fruits aver-

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aged smaller than comparable normal Washington navels, with a more pebbled rind texture and a smaller navel opening. The flesh was softer and more juicy than in normal fruits, and acidity averaged two- to four-tenths percentage points higher during the main part of the harvest season. Because of high acidity, the fruit has often failed to meet legal maturity standards early in the season. This variant is believed to have originated from a budstick from a normal Washington navel tree (Frost selection) of nucellar origin. The budded variants were not immediately detected, since vigorous growth is typical of propagations from nucellar selections. A widespread top-working program was carried out to eliminate the mutant.

Bud Variation in the Satsuma.—Satsuma is a citrus group in which bud variation has been very important. Extensive studies in Japan by Tanaka (1932) over a period of many years showed that numerous varieties have arisen by limb variation. At least three distinct varieties, the Owari, Ikeda, and Zairai, seem to have been established in various areas of Japan by the time the studies began in about 1909. A fourth type, called Wase, was also being grown, and this was traced back to a group of four source trees, from which it became known as Kuwano Wase.

In some orchards of Kuwano Wase, further bud variations were found; these took the form of reversions to the Owari fruit type. At about the same time, it was shown that a bud variation branch on a known Owari tree had fruit characters essentially identical to those of Kuwano Wase. This was the Higuchi Wase. Such evidence led to the conclusion that Kuwano Wase is a bud sport from Owari. Many other bud variations classed as Wase types were identified on Owari, Ikeda, and Zairai trees. These varied among themselves, although early maturity is apparently a common feature. At least part of their differences could be ascribed to differences in the parent varieties.

New variations have continued to be reported in Japan. Thus, Yamaguchi and Yakushiji (1960) described two early satsuma sports, the Matsuyama and the Tichima, both from Owari. Iwasaki *et al.* (1962) described a strain test of seven early satsuma types, carried out from 1939 to 1961. Five of the selections previously described by Tanaka were included, and two newer ones, Iseki and Mikkabi-10. The Miyagawa was judged best; vegetative reversion was observed in a Suzuki selection during the study.

The mechanisms involved in satsuma bud variation are uncertain. It is not easy to ascribe all the correlated changes seen in a mutant type to single-gene mutation, although this is not impossible. The tendency of the Kuwano Wase to frequent reversion is suggestive of a shift in germ-layer composition of a chimera, although Tanaka did not favor this explanation. All sports classed as Wase types may not be due to the same genetic change. All seem to have been selected for earliness, but Iwasaki *et al.* (1962) report that fruit shape, coloring time, susceptibility to disease, and other characters are not the same among the variants.

Nakamura (1929), in cytological studies of some of the Wase type variations, found no differences in the chromosome number ($2n = 18$) and reported that meiotic behavior seemed normal. Small changes in chromosome structure, however, are difficult to detect in citrus.

Frost, Cameron, and Soost (1957) reported a long-term study of nucellar-seedling selections of satsuma, which were judged to show effects both of nucellar embryony and of genetic variation (table 5–4). Three selections were identified from one seed-parent tree. Selections A and B were obtained repeatedly from seeds of fruits from different branches on the parent tree, and a third selection, C, arose from a seed from the same branch which produced a seedling of A. Budded trees of A and B both showed larger tree and leaf size, greater yields, and more alternate bearing than trees from the seed-parent budline; these differences seemed to be effects of nucellar embryony. Fruit coloring was earlier in these two nucellar selections, and soluble solids were higher throughout the season. Trees of A were more open and had flatter fruit, which usually colored earlier than those of B. Selection C was more erect, with later-maturing, thicker-rinded fruit.

It is almost certain that all of these selections were of nucellar origin; the seeds came from fruits from controlled pollination by parents very unlike satsuma. Indexing tests indicated that the tristeza virus was not involved. Selections A and B probably existed as shoots on the seed-parent tree as a result of bud variation. Selection C may have occurred in the same manner, or it may have arisen in nucellar tissue; C, at least, is a genetically distinct type (a strain in the sense of Shamel, Scott, and Pomeroy, 1918). The seed parent appears to be Owari, which gave rise to many of the variants in Japan. Horticulturally, selection A has been superior to B and C, and to the parent.

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Table 5–4

CHARACTERISTICS OF TWO NUCELLAR-SEEDLING SATSUMA SELECTIONS AND THEIR SEED-PARENT BUDLINE.

	Parent Budline	Nucellar Selection A	Nucellar Selection B	P Values* Parent Versus Nucellar A or B	P Values* Selection A Versus B
Trunk cross-section area of 15-year-old trees in 1951 (sq cm)	87.0	116.5	115.0	<0.02	NS
Length of leaf lamina in 1936 (mm)	87.8	105.5	100.0	<0.01	0.14
Length of leaf lamina in 1956 (mm)	90.2	111.0	105.7	<0.01	0.12
Fruit shape index (average of 9 years)	1.104	1.131	1.103	A 0.03 B 0.90	0.03
Fruit yield from 1947–55 (lb/tree/yr)	80.0	137.0	109.0	<0.02	<0.01
Per cent of fruit well colored at early maturity from 1947–55	60.0	93.0	88.0	<0.05	0.15
Per cent of soluble solids in juice at early maturity from 1947–55	12.1	13.4	13.2	<0.01	NS

Source: After Frost *et al.* (1957).
* Tests of the significance of the difference of two means.

Chimeras

Composition, Behavior, and Terminology.—A plant chimera is a combination of tissues of two or more genetic constitutions in the same individual plant or part of a plant (fig. 5–10). The term chimera is not applied to ordinary budded and grafted plants, but is restricted to forms in which the combined genetic types (components) grow together, side by side, in the same part of the plant. These components, like the stock and scion of a budded tree, maintain separate genetic constitutions in their respective cell lineages, but their close association results in mutual physiological influence and often compels compromise between conflicting growth tendencies. As a rule, the components of a chimera no doubt differ in chromosome or gene constitution; occasionally, they may differ in plastid constitution only, as in some green-and-white variegated forms.

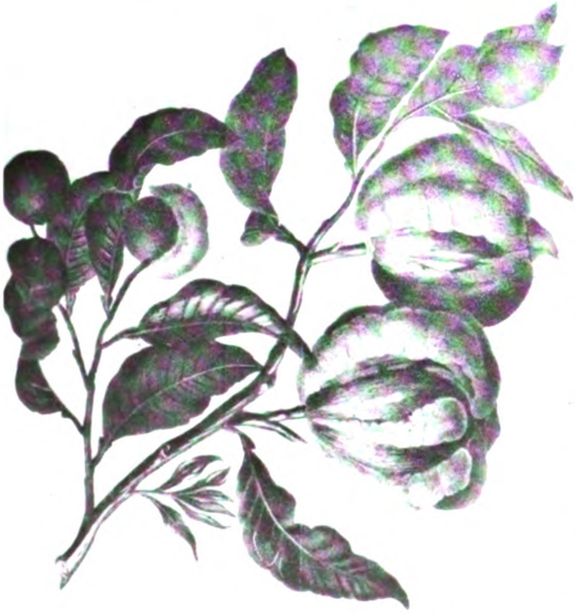

Fig. 5–10. Bizzarria (a synthetic chimera) of citron and sour orange. (After Tozzetti, from Nati, 1929 edition, edited by Ragionieri.)

The term graft hybrid correctly refers to chimeras resulting from graftage. There is little evidence in higher plants for the existence of hybrids produced by actual fusion of somatic cells of different genetic types.

Chimeras are classified according to the relative positions of the components. If one component forms an outer covering surrounding an inner core of another component, the combination is a *periclinal* chimera (Jones, 1934). If one component does not surround the other, but both extend to the surface or the center of an organ, the chimera is *sectorial.* If the arrangement is externally sectorial, but one component does not extend to the center of the shoot, the chimera is often called *mericlinal* (Jorgensen and Crane, 1927). If the two genetic types are irregularly mixed throughout the plant, as in some forms of variegation, they form a *mosaic* or *mixed* chimera.

Chimeras may be classified also on the basis of their origin (Frost, 1926*a*). If a chimera is produced by growth from tissues of two varieties or species, at a bud or graft union, it is *synthetic;* if it is produced by genetic change in a cell or cells originally like the rest of the plant, it is *autogenous.*

All these kinds of chimeras seem to occur in citrus trees; in view of their importance in relation to bud variation and variety improvement, their characteristics are discussed in detail below.

Developmental Relationships in Chimeras.—Chimeras can be analyzed on the basis of the arrangement of the component genetic types in leaf, stem, and flower tissues, together with the ways in which the various parts are developed from the meristems, or growing points, of the plant. One growing-point cell layer, or *primary histogen* (Dermen, 1945) may produce one or more layers of the mature tissue; in the fruit, tissue of superficial origin may be deeply buried.

In older studies, evidence for chimeras was usually based on the morphological and histological appearance of the tissues. Some of the best-known cases in citrus can be recognized in this way. Differences in chromosome number, when they occur, provide a much more accurate means of identifying the cell lineages. If the epidermis, for example, carries a tetraploid complement of chromosomes, while the hypodermal tissue carries the diploid number, tissue derivation can be determined in the developing parts by chromosome counts, and often by cell size. During the past twenty-five years, this method has been used in several plant studies. In *Datura,* extensive investigation has shown that three primary histogenic layers are regularly present in the apical meristems, and that these layers give rise to certain tissues of the stem, leaves, and fruits (Satina, Blakeslee, and Avery, 1940; Satina, 1945).

Dermen (1947, 1953*a*, 1953*b*) employed the same method to trace the origin of tissues in cranberry, apple, and peach, where three histogenic layers are also the usual basis for development. In many of these fruits, and in *Datura,* the polyploid condition was induced by colchicine.

The number of histogenic layers and the regularity of their behavior are incompletely known in most families of plants. A review by Gifford (1954) showed that in a majority of the dicotyledons studied there were two primary outer layers and presumably one core layer, while in monocotyledons only one outer layer was present in many cases. Citrus appears to have three histogenic layers.

In dicotyledonous plants, the epidermis is regularly developed from the outermost growing-point layer (the dermatogen, or Layer I): Layer II produces a marginal zone of leaf tissue, or even all the leaf parenchyma inside the epidermis, as well as the gametes and at least part of the associated tapetal and nucellar cells. In certain green-and-white chimeras a green-producing Layer II usually produces a larger part of the leaf tissue than does a white-producing Layer II (Rischkow, 1936). In some exceptional cases Layer I, instead of Layer II, gives rise to the gametes (Jones, 1934, p. 42). Differences in growth-rate tendencies between the components of a chimera may affect the regularity of tissue differentiation and the stability of the chimeral pattern.

Sectorial chimeras are generally unstable; in the formation of lateral buds, one component is often eliminated or a periclinal arrangement may be formed. The conditions producing mosaic chimeras include: (1) irregular assortment of green and colorless plastids in cell division; (2) frequent genetic change within cell nuclei; and (3) unbalanced growth relations in a periclinal chimera, such that one component frequently takes the place of another. In citrus forms where there seems to be frequent genetic variation within cells, the variable phenotype may depend on several grades of chromosome constitution, apart from changes in the relationships of tissue components.

Synthetic Chimeras.—In early experiments, Winkler (1908) grafted tomato onto its relative, the nightshade, and nightshade on tomato, after which he cut off the grafted plants at the union. Sprouts developed from adventitious buds, and occasionally one arose at the line of the union so that one side of the shoot was tomato and the other nightshade, constituting a sectorial chimera (fig. 5–11). Intermediate forms were also obtained, which when analyzed by chromosome counts and seedling characters proved to be periclinal chimeras (Winkler, 1910; Baur, 1910). These chimeras had one or more outer layers of tomato tissue and a core of nightshade, or vice versa (fig. 5–12). Where synthetic chimeras are so formed, by the combination of different species, identification of the chimeral condition is fairly easy. Forms capable of somatic separation into two types having leaves or fruit clearly of two species have probably been produced by graft synthesis.

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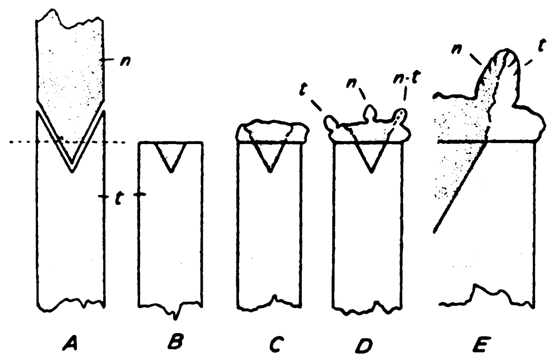

Fig. 5–11. *A-E*, stages in the production of a synthetic sectorial chimera by Winkler's method. Grafted stem (*A*) was later cut off at dotted line. *n*, nightshade; *t*, tomato; *n-t*, chimeral bud (shown enlarged at *E*). (After W. N. Jones, 1934.)

The citrus bizzarria.—Several freakish citrus forms, known in Italy as bizzarria, are evidently synthetic chimeras. The bizzarria of Florence was described by Nati (1674, 1929); it had branches, leaves, and fruits partly like citron, partly like sour orange, and mainly intermediate or mixed (fig. 5–10). Nati concluded, mostly from testimony of the gardener under whose care the bizzarria had originated, that it probably arose at the union where a citron bud had been inserted on a sour orange seedling—the bud failing to grow at first, but a mixed shoot coming out later. Strasburger (1907) concluded that this bizzarria did not result from actual fusion of somatic cells, since it had the same chromosome number as the parent types, not twice as many. Tanaka (1927*b*) studied Nati's bizzarria, and concluded that it is primarily a periclinal chimera, with a core of citron and outer layers of sour orange, the sectorial structures resulting from irregularities in histogenic-layer behavior; most of the rind is superficially sour orange, with yellowish streaks where the citron core has emerged, and occasional wider sectors of thick citron rind. The instability of this chimera may be due to the more vigorous growth of the inner component.

Strasburger (1907) summarized accounts of other citrus bizzarria, including one of citron and sweet orange that was in existence before 1644, when the bizzarria of Nati is said to have originated. Savastano and Parrozzani (1911) described as "natural hybrids" three bizzarria which presumably were synthetic periclinal chimeras. Brown (1918) described a tree that appeared to be a lemon-over-orange chimera, and Uphof (1935*b*) described a tree that bore intermingled fruits of sweet and sour orange. Intermediate fruits were not observed.

In Japan, two citrus forms considered to be synthetic periclinal chimeras have been in existence for some fifty years (Samura and Nakahara, 1928; Takahashi, 1962). The Kobayashi Mi-

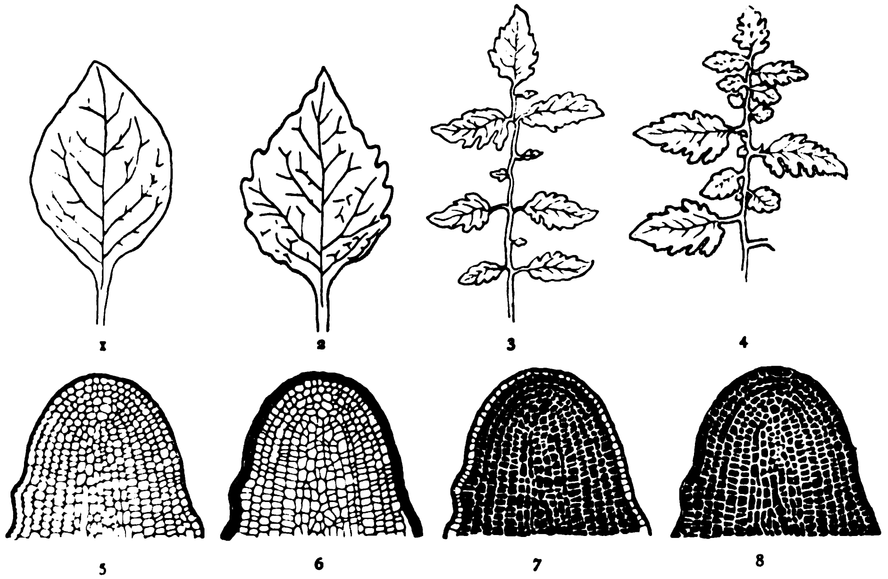

Fig. 5–12. Leaves and stem growing points of nightshade (1,5) tomato (4,8) and chimeras between them. Leaf 2 and growing point 6 have an epidermis of tomato with inner layers of nightshade; 3 and 7 have the reverse combination. (After Winkler, 1935.)

kan is recorded as having arisen at the junction where a satsuma scion was grafted onto Natsudaidai. This scion was accidently broken off at its base, after which two adventitious buds emerged, one of which grew into the original chimeral tree. This chimera produces fruit with rind like Natsudaidai, but with flesh like satsuma. It is seedy, and seedlings from one fruit were reported to be Natsudaidai (Dr. Yuishiro Tanaka, unpublished). In this chimera, histogenic Layer II appears to be genetically Natsudaidai, while Layer I should be satsuma.

Kinkoji Unshu arose in a similar manner, in about 1912, after satsuma scions had been topworked onto Kinkoji (*C. obovoidea* Takahashi). A branch was later observed which produced fruits having outer parts like Kinkoji and inner parts like satsuma, with the fruits bearing some seeds. Both of these chimeras have been maintained in Japan.

Casella (1935) described and showed photographs of a tree of sweet lime budded on sour orange, which produced a vigorous chimeral branch at the bud union. This branch produced some fruits similar to lime, some similar to orange, and some chimeral fruits with rind sectors of both types. Casella reported that seeds and juice vesicles in these fruits and fruit sectors corresponded in character to the respective rind types. He stated that six-year-old seedlings from seeds from orange-like fruit sectors had characters of sour orange, but that those from lime-like sectors also had sour orange characters. Casella concluded that the variant branch was a synthetic chimera.

Autogenous Chimeras.—Baur (1909) discovered that certain variegated forms of *Pelargonium* (garden "geranium") are white-over-green or green-over-white periclinal chimeras. In later studies, such as those of Bateson (1916) and Chittenden (1927), other herbaceous plants were found to be periclinals. Since the varieties concerned are propagated by cuttings, not by graftage, these chimeras no doubt originated by some change in cells of the parental clones.

The components of such chimeras can often be identified by one of the following methods: (1) in green-and-white combinations, the components are visible by inspection in parts normally green; (2) in citrus, a component present as the subepidermal layer which produces the nucellus should appear in all histogenic layers of the derived nucellar seedlings; and (3) components with differing chromosome numbers can be identified during cell division, in certain tissues. A component present as an inner layer, from which adventitious roots arise, can sometimes be detected by obtaining whole plants from such roots. In some bud variation forms, root cuttings of this kind give the parental type that produced the variation; thus, certain thornless varieties of blackberry produce thorny plants from root cuttings.

Many of the mutant types that arise in growing-point cells of *Citrus* must be inferior to the parental type in vigor and are soon crowded out. Occasionally, however, a variant type is noticeable because it has come to occupy an extensive region in one or more cell layers. If a bud-variation shoot contains only an outer layer of a new type, it may lose this type through emergence of the inner type, or it may lose the inner type by the reverse process. Since chimeras are subject to such changes, a bud variation is not necessarily transmitted to all trees budded from it.

Variant sectors of rind occur commonly but very irregularly on fruits of many varieties of *Citrus*. Certain characters may be conspicuously changed, such as thickness, smoothness, and color of rind, size of oil glands, and color of pulp; often, several rind characters are changed together. When such changes occur only occasionally within a tree or clone, they presumably represent separate new occurrences of mutant types in cells of the young fruits.

Forms showing leaf variation give valuable evidence on the behavior of chimeras, because of the ease of recognition of the cell types concerned. In normally green seed plants, the epidermis is without green color, except that usually there are chloroplasts in the stomatal guard cells. If a mutant subepidermal layer of the growing point also produces non-green tissue, but the layer within it produces green tissue, the leaves commonly have a white marginal zone and also have at least one colorless layer of cells under the epidermis in the green central portion of the leaf (fig. 5–13, *E-H*). In contrast, if the subepidermal histogen is green-producing, but the third layer is not, the marginal zone of the leaf has normal green color, while the central portion is pale because it has a white core under one or more layers of green cells (fig. 5–13, *A-D*). Irregularities may produce a shoot of the reverse form or one which is entirely green or entirely white. For examples in *Poinsettia* and in the privet, see Dermen (1950). The patterns of variegated leaves in different species indicate that tissue zones are formed from the growing point in a

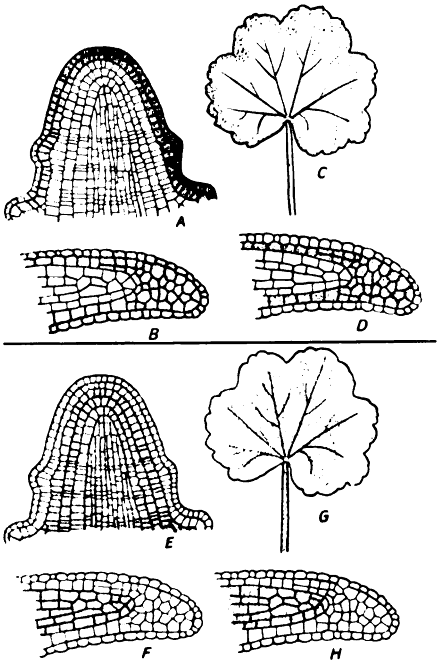

Fig. 5–13. Cell layers and distribution of chlorophyll in green-over-white (*A-D*) and white-over-green (*E-H*) chimeras in a plant in which the second and third histogenic layers form the mesophyll of the leaf. In the left column, the shading indicates *genetically* green and non-green cell layers of stem growing points *A* and *E* and of leaf margins *B* and *F*. In the right column, the shading indicates the *actually* green tissue of the leaves, the epidermis being colorless in all forms. In *C* the central region is in reality pale green, because of the green subepidermal layer. (After W. N. Jones, 1934.)

much more regular way in some kinds of plants than in others, citrus chimeras being especially irregular.

White-over-green chimeras.—Shamel *et al.* (1920*a*; 1920*b*) described variegated bud-variation strains of Eureka and Lisbon lemon. Another form somewhat similar in type of leaf variegation, designated as Variegated Pink lemon, was discovered as a whole tree (Shamel, 1932). It has leaves with an irregular white marginal zone, the white regions often being much dwarfed (fig. 5-14). The upper sides of the leaves show as many as four different grades of green, and the lower sides two or three, the upper and lower patterns being mainly independent. The immature fruits have narrow longitudinal stripes of thin whitish and normal green rind, the green becoming more abundant toward the apical end. Mature fruits are yellow, slightly ridged, and have pink flesh. The bark of the young shoots is usually pale, with longitudinal stripes of deeper green commonly arising at the leaf axils. In each growth cycle, the amount of green in bark and leaves usually increases from base to apex of the shoot. Occasionally a shoot is completely white. The characteristics of this chimeral variety are still regularly expressed in a budded tree now more than thirty years of age at Riverside, California. The variety may not be a simple white-over-green form; mutations in plastids, followed by plastid segregation at somatic cell divisions, might account for its chlorophyll patterns.

In some light-margined citrus forms, the non-green areas may be creamy white; in others they are light yellow. In the Imperial orange, grown at Riverside since 1914, the leaves are variegated similarly to those of the Variegated Pink lemon. Fruits are orange-yellow, with narrow, thicker stripes of reddish-orange. These characters appear irregularly throughout the tree, year after year. Small populations of seedlings of this variety, from open pollination, have been entirely white-leaved and soon die. Seedlings from a variegated, white-over-green Eureka lemon described by Shamel (1932) also produce white leaves. This behavior supports the assumption that germ Layer II is producing both the nucellus and the white leaf tissues in the seed-parent tree.

In such chimeral forms, both Layer II and Layer III seem to be participating in the formation of the leaves. Layer III apparently has normal chlorophyll-producing capacity; the formation of white leaf tissue from Layer II seems to undergo rhythmic changes with the growth cycles. Completely white shoots suggest that Layer III is occasionally lost from a growing point, but the white-producing Layer II is very seldom lost.

Green-over-white chimeras.—A budded tree of a variegated Lisbon lemon (CRC 2519) grown at Riverside before 1930, had leaves whose upper sides showed central areas of light green, surrounded by marginal zones of normal, darker green. A few leaves seemed entirely normal. The lighter areas were variable in size and shape, and sometimes showed at least two tints lighter than normal, yet the variation was less than in the white-over-green lemons described above. The lower sides of the leaves only occasionally showed light areas. No completely white areas were found, and the bark of young shoots seldom had light streaks. This lemon was evidently a green-over-white chimera, but the white core component did

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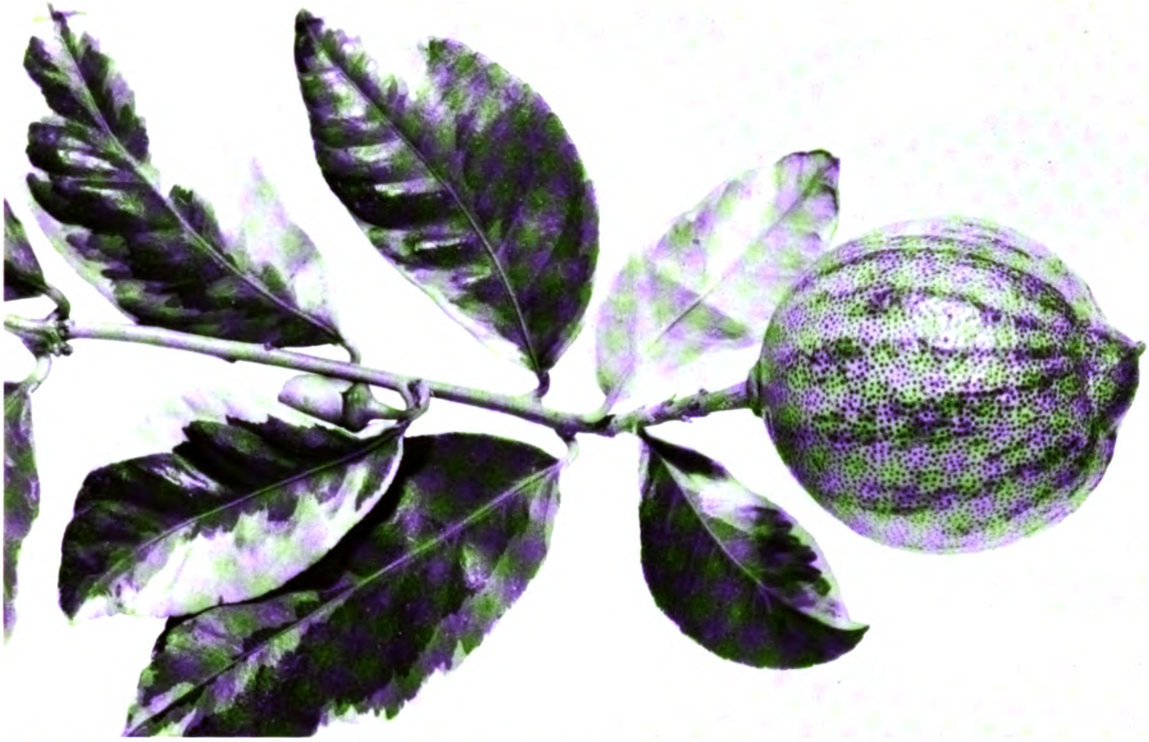

Fig. 5–14. Variegated Pink lemon (CRC tree 12A,1,10); apparently a form of white-over-green periclinal chimera.

not emerge to produce subepidermal tissue.

An instance of complex instability is evidenced by a clone of variegated sour orange (CRC 622) budded at Riverside in 1914, from a seedling which showed leaf variegation when about 18 inches high. The original budded tree is still living. Most of the foliage has been normally green but some leaves are variegated (fig. 5-15) similarly to those of Lisbon lemon just described. Occasional leaf areas appear white, but these are often bordered by a green zone along the leaf margin. Some twigs with much leaf variegation have bark with whitish stripes. Mature fruits (fig. 5–16) usually show a normal, deep orange rind color, but they may have a narrow stripe or wider sectors of thin, smooth, lemon-yellow rind; rarely, a fruit is mainly yellow and of small size. This sour orange chimera shows some characteristics of a green-over-white type, as if a white layer were occasionally emerging to form subepidermal tissue; however, seedlings, including both nucellar and zygotic ones, continue to show the chimeral condition. In 1963, thirty-one young seedlings were grown from open-pollinated seed from variegated fruit by D. A. Cole, Jr. Seventeen probably nucellar and three seemingly zygotic plants all showed some leaf variegation of the same type as in the seed parent. Eleven entirely white-leaved seedlings died very young. This chimera seems to be due to an unstable genetic factor; the white seedlings may originate from already mutated cells, while in predominantly green seedlings mutations occur during growth. Plastid segregation could be involved.

Other autogenous citrus chimeras.—Other bud-variation strains give indication of chimeral constitution, without leaf variegation. The Golden Buckeye navel orange (Shamel, Pomeroy, and Caryl, 1925) is one of these; it appears to have an inner layer similar to Washington navel, surrounded by a subepidermal layer producing a smoother, more yellowish rind. The rind shows narrow longitudinal stripes and ridges of rougher, reddish-orange tissue which seems identical to that of the Washington. Fruit shape tends to be more elongated than with the Washington; the pulp is firmer and sweeter, with a different aroma, and a navel opening is less commonly present. An occasional branch produces fruits which seem to be entirely Washington.

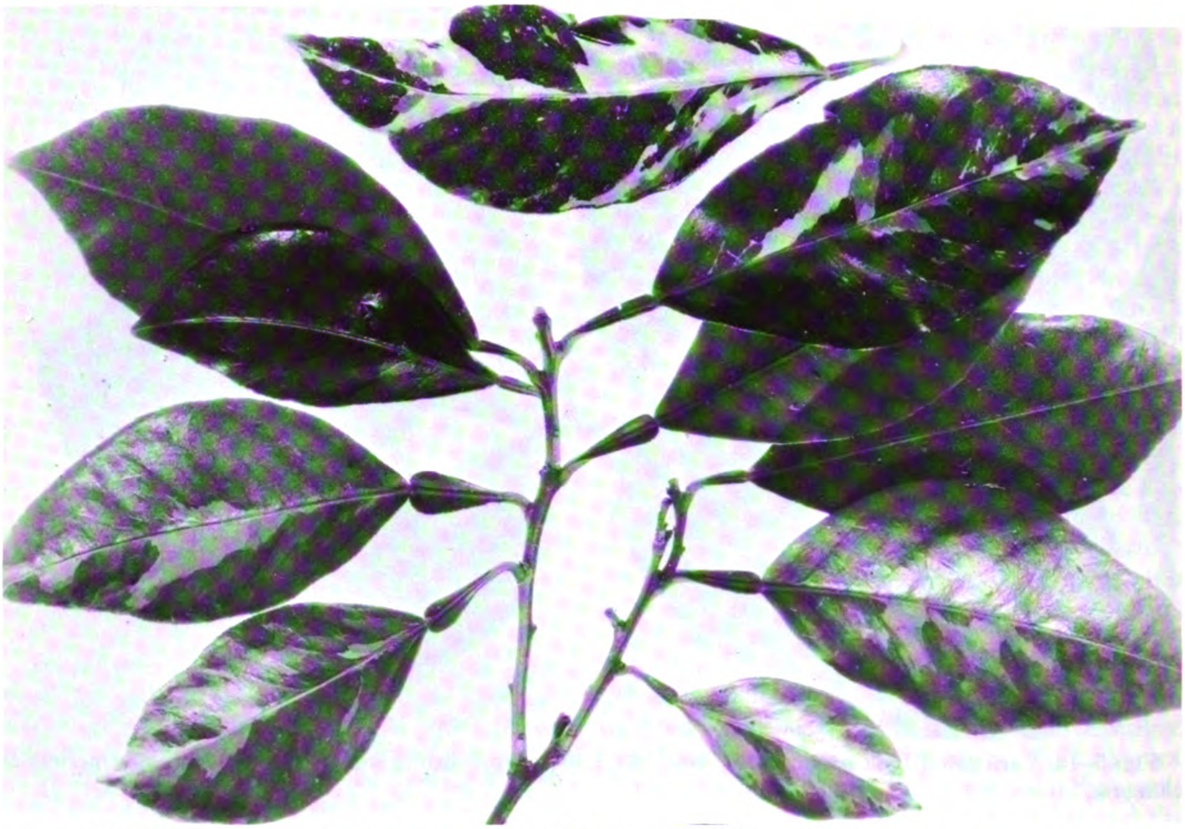

Fig. 5–15. Leaves of a variegated sour orange (CRC tree 8A,16,23) with characters of a green-over-white chimera, but perhaps involving an unstable genetic factor.

The difference in the pulp of the Golden Buckeye may be explained by the fact that in citrus both the juice sacs and the outer tissues of the rind seem to be formed by outer histogens. If only Layer III is of the Washington type, Washington characters should not appear in the juice sacs. If this histogen frequently emerges to form stripes on the rind, after the development of the fruit is well underway, the stripes would not necessarily be correlated with underlying pulp characters.

Shamel, Pomeroy, and Caryl, (1929) described other citrus types which seem to be periclinal chimeras. The fruit rind of the Seamed form of the Washington navel shows narrow, longitudinal grooves, which may be caused by the emergence of a mutant Layer III. The Dual selection of Washington navel has patches of smooth and rough rind, but not regularly in longitudinal sectors. The smooth areas sometimes form an equatorial zone, between polar caps of rough rind. These patterns suggest a chimera in which the inner component emerges frequently during the earliest stages of ovary formation, but seldom thereafter.

Frost and Krug (1942) described two cytochimeral forms obtained from a bud variation branch on a hybrid mandarin tree. Chromosome counts of root tips, pollen mother cells, and leaf and stem primordia indicated that three histogenic layers were present and that the ploidy of Layers I, II, and III in the two forms was 2–4–4 and 2–4–2. Root tips from stem cuttings were $4n$ from the 2–4–4 plant and $2n$ from the 2–4–2 form, as would be expected if such roots are produced from Layer III. Growth habit was lower and broader in the 2–4–4 plant than in the 2–4–2 form. The origin of inner leaf tissues was uncertain; cell divisions in layers other than epidermis showed the $4n$ condition in both plants, suggesting that only Layer II is involved, but this is contrary to evidence from green-and-white leaf chimeras already discussed.

Evidence that the pink-fleshed Thompson

and Foster grapefruits are periclinal chimeras has been obtained by Cameron, Soost, and Olson, (1964). These varieties arose, respectively, from the white Marsh and the white Walters grapefruits, by somatic limb variation. In the Foster, the rind shows color, but in the Thompson it does not. By further somatic variation, the Thompson has repeatedly produced red-fleshed forms such as Henninger's Ruby and Webb's Redblush (Waibel, 1953), which appear to be identical. In all of these colored varieties, lycopene and carotenes are the coloring compounds (Khan and MacKinney, 1953). Nucellar seedlings of Thompson, tested over several seasons in California, show no pink or red color in their fruits and appear to be identical to the Marsh. Nucellar seedlings of Foster, produced both in Texas and in California, have fruit with red flesh color like the Redblush and continue to show color in the rind. On the other hand, nucellar seedlings of Ruby, Redblush, and others of the red-fleshed group reproduce the fruit color of their seed parents without change. It appears that a mutant color factor is present in the Thompson in histogenic Layer I; this layer provides at least part of the cells of the juice vesicles (see chap. 1, p. 15) but should not form subepidermal rind tissues. The factor is not present in Layer II, from which nucellar embryos apparently arise. In the Foster, a factor must be present in Layer II, but perhaps not in Layer I. Nucellar seedlings of Foster, and those of the Redblush group, should carry the factor in all layers. Occasional sports to Redblush, produced on Thompson trees, would then represent the substitution of a cell lineage from Layer I into Layer II, and possibly also into Layer III. The fact that the juice vesicles are pink in Thompson and Foster and red in Redblush may be related to interaction among the tissues involved (Purcell, 1959).

An unusual pink sport of the Shamouti orange, called Sarah, also contains lycopene and carotenes, rather than the anthocyanins commonly found in pigmented oranges (Monselise and Halevy, 1961). As in the red grapefruits, these carotenoids occur in the rind, septa, and juice vesicles, but apparently not in the filtered juice.

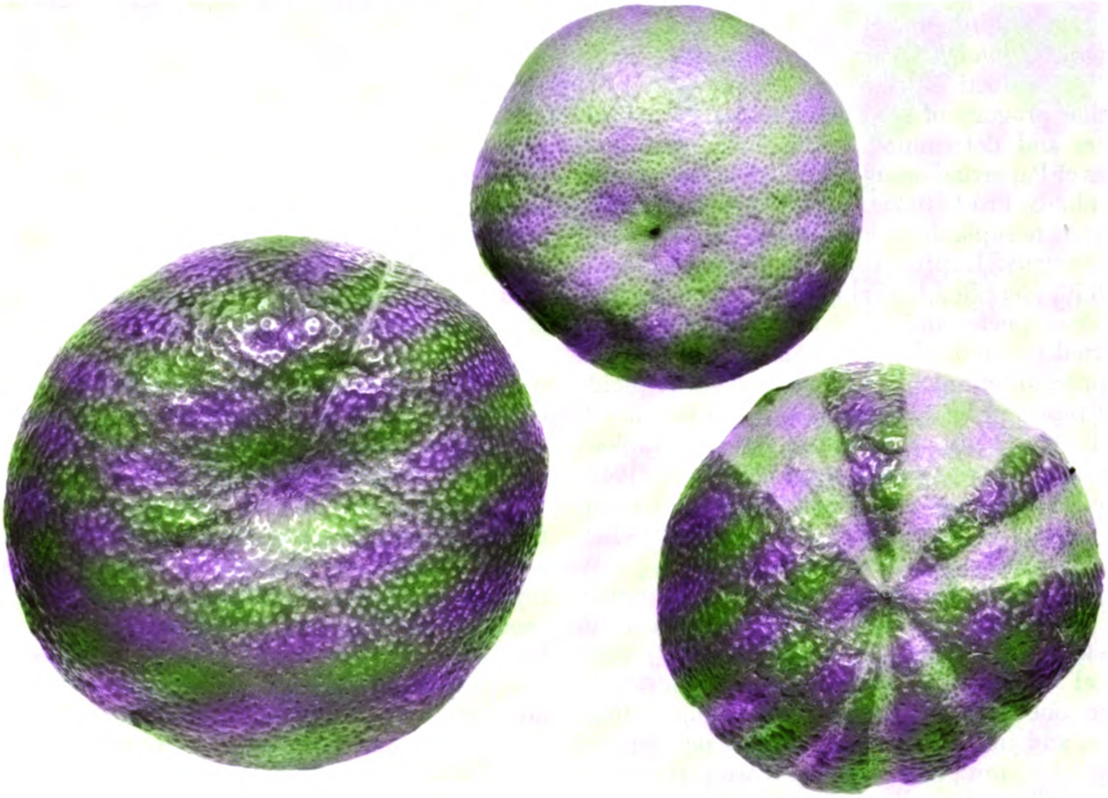

Fig. 5–16. Fruits of a variegated sour orange (see leaves in fig. 5–15). Normal rind areas are orange and depressed stripes are yellow. The abnormally small fruit at top is almost entirely yellow-rinded.

Dr. K. Mendel (personal communication) has stated that the Sarah shows typical characters of a chimera. Some branches bear fruit which is essentially Shamouti in type, while others carry fruit with pink color in the albedo and segment membranes. One budded tree shows further color variation in the juice vesicles.

Sinclair and Lindgren (1943) found that cyanide fumigation at an early stage of flower-bud development often produced abundant and varied sectorial rind variations. Although the variant characteristics indicated or simulated genetic change, perceptible effects were limited to the immediate crop season; shoot buds appeared to be unaffected.

Polyploidy

Diploidy is the general rule in *Citrus* and its related genera, the somatic chromosome number being 18 (see chap. 4). However, forms with increased chromosome numbers have been identified or produced by several workers. Such forms are useful in genetics and breeding. Longley (1925) was the first to report that a form established in nature, the Hongkong wild kumquat (*Fortunella hindsii* Swing.), was tetraploid. Frost (1925) described "thick-leaved" forms among the nucellar progeny of seed parents used in hybridization and determined cytologically that such plants of Paperrind orange and Lisbon lemon were tetraploids. Frost (1926*a*, and cited in Krug, 1943) reported tetraploids in five species of *Citrus* and in *Poncirus*. Lapin (1937) in Transcaucasia, U.S.S.R., identified tetraploid seedlings in a series of *Citrus* species and relatives. Nakamura (1942) reported two tetraploids, the Shikinarimikan and an apparent tetraploid Sampson tangelo. Triploids were reported by Longley (1926), Lapin (1937), and Bacchi (1940). A hexaploid and a pentaploid have also been reported (Lapin, 1937; Krug, 1943) as well as occasional aneuploids (Krug and Bacchi, 1943). Some of these forms are discussed below and in the section on breeding.

Tetraploids.—Among many citrus varieties used for breeding at Riverside, California, from 1914 through 1916, nearly all seed parents (except several which gave few nucellar seedlings) produced one or more tetraploid seedlings (Frost 1926*a*, and unpublished). About 2.5 per cent of some 3,600 nucellar progeny were tetraploid. Open-pollinated seedlings from various hybrids, including the hybrid mandarins Kara and Kinnow, and from highly apogamic oranges, including the Washington navel, also gave seedlings that from their resemblance to cytologically determined forms are almost certainly tetraploid. In Russia, tetraploids identified by Lapin (1937) occurred in eight *Citrus* species or varieties in percentages ranging from less than 1 to 5.66. Lapin also found thirteen tetraploids among 353 known nucellar seedlings of *Poncirus trifoliata*.

In Italy, Russo and Torrisi (1951*a*, 1951*b*) obtained spontaneous tetraploids in *Citrus limon* (L.) Burm. f. and *C. aurantium* L. Furusato (1953*a*) found a low percentage of tetraploids among seedlings of four *Citrus* species. Although the leaves were thicker and darker green than in sister diploids, he stated that the tetraploids within a variety were uniform, having the features of the seed parent, and were presumably nucellar autotetraploids.

Origin of autotetraploids.—Spontaneous tetraploids appear to occur almost entirely as nucellar seedlings. The older Riverside tetraploids that have been cytologically verified are of this type, as shown by the absence of pollen-parent characters after cross-pollination and by uniformity within a variety. The tetraploids studied by Lapin included some of known nucellar origin and none of proven sexual origin. Nearly one fourth of the early Riverside tetraploids came from seeds which also gave diploid seedlings, and nearly all these seeds came from fruits which also gave other diploid seedlings. It is thus evident that doubling of the chromosome number occurs repeatedly in the parent trees, often in the tissues of the ovule or ovary. Doubling does not often normally occur (or at least the polyploid cells seldom persist) in the young embryo, since tetraploid hybrids have seldom been reported from crosses of diploid parents. Triploids do occur spontaneously from such crosses, indicating that one gamete was already double at fertilization.

Tachikawa, Tanaka, and Hara, (1961) reported the occurrence of thirteen tetraploids from a total of eighteen seedlings from crosses of the reportedly monoembryonic, diploid *Citrus tamurana* Hort. ex Tan. and *C. iyo*, Hort. ex Tan. pollinated by tetraploid Natsudaidai. The mechanisms responsible for these unexpected tetraploids are undetermined. (See also page 356.)

Tree and fruit characters of autotetraploids.— Effects of tetraploidy in citrus can be rather accurately evaluated in nucellar selections, since meiosis and gene segregation are not involved. Except for possible bud variation, such

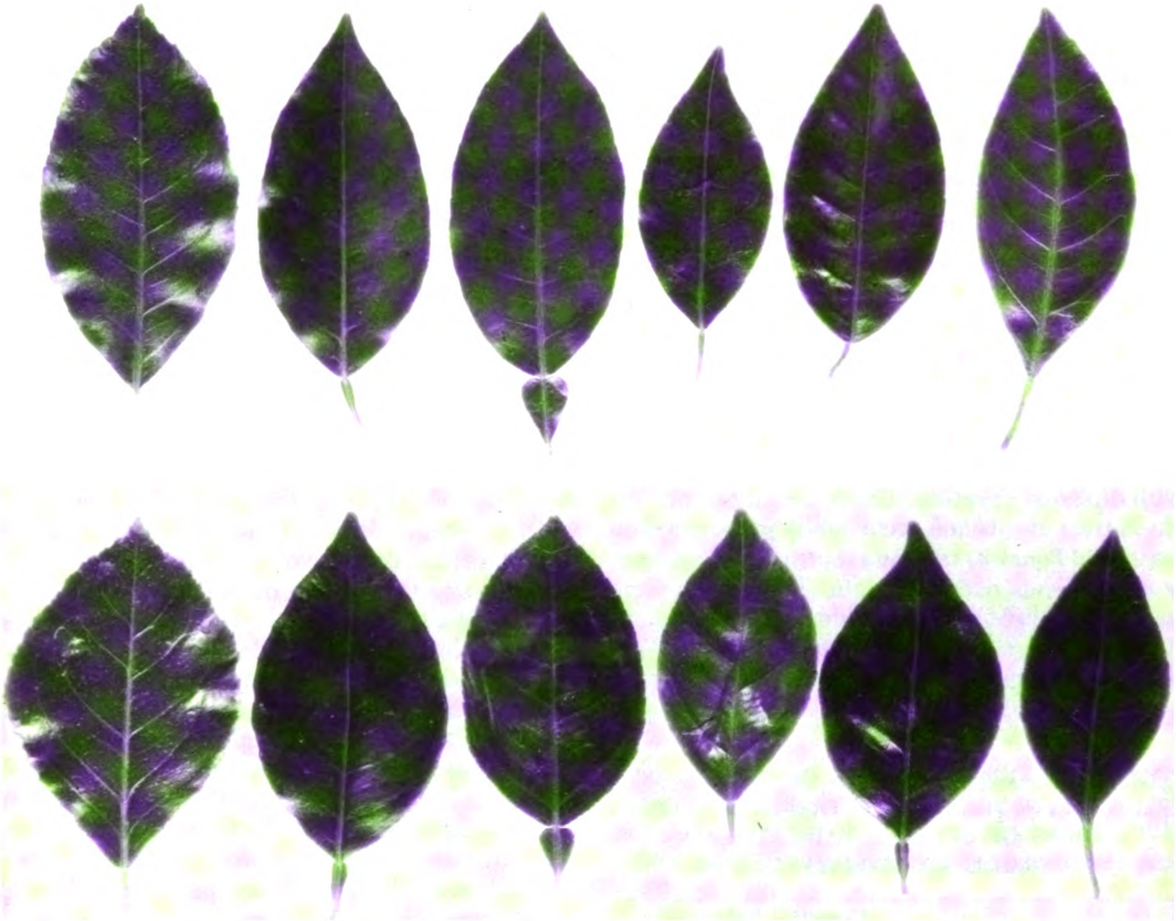

Fig. 5–17. Diploid (top row) and tetraploid (bottom row) leaves of six citrus varieties. Left to right: Lisbon lemon, Paperrind orange, Hall's Silver grapefruit, Dancy mandarin, King mandarin, Owari (?) satsuma. Tetraploid leaves are usually broader and thicker than diploids.

tetraploids differ genetically from sister nucellar diploids only in having the doubled number of chromosomes. The following descriptions apply to tetraploids studied at Riverside, California. Tetraploid leaves (fig. 5–17) are considerably broader in proportion to their length than diploid ones, and considerably thicker; leaf color tends to be darker. The wings of the petioles are usually broader, and in some varieties they are often fused with the leaf blade.

In young seedling selections and their immediate budded progeny, thorniness is at least as pronounced in tetraploids as in diploids, and the thorns are stouter. As the selections become older from seed, new growth in tetraploids remains more thorny than in diploids. Growth is slower in tetraploids; vigorous shoots are less common, and the top is smaller, less erect, and more compact.

Tetraploids are slower than diploids in beginning to bloom and set fruit, and are generally much less fruitful. Among the Riverside selections, only the tetraploids of the Lisbon lemon and of some grapefruits have been highly productive, often giving heavy yields, while a tetraploid Willowleaf mandarin has been the least productive of all, usually bearing no fruit. In the latter tetraploid, excessive dying of the outer twigs occurs, possibly from sunburn. A tetraploid Dancy tangerine has been very susceptible to dying and shelling of large areas of bark, with associated death of large parts of the tree. Dying of branches also has been severe in the Ruby orange and Lisbon lemon.

Many Riverside autotetraploids have now

been maintained as budded trees or original seedlings for over thirty years. Table 5–5 lists thirteen of the selections and indicates their present condition. The grapefruit tetraploids have been strikingly more vigorous and healthy than any of the other varieties. While some propagations of King, Dancy, and Lisbon have died, the budded trees of Imperial and Royal grapefruit are about equal in size and vigor to adjacent diploids. Where tetraploids have been established on both rough lemon and sour orange rootstock, long-term vigor has been much the better on the sour orange. Tetraploids on their own roots have often been smaller in size than when budded on a rootstock. Furusato (1953*a*) obtained tetraploid seedlings of four *Citrus* species, and found that the main root was thicker and the number of lateral roots fewer than with diploids. Furusato (1953*b*) also described orchard trees of satsuma accidentally propagated on tetraploid *Poncirus trifoliata* rootstocks of evidently spontaneous nucellar origin. These trees were smaller and yielded less fruit than trees on diploid stock. At Riverside, a nucellar tetraploid *Poncirus* seedling has grown vigorously for many years. However, in an experiment with seedlings of this tetraploid as a rootstock, several *Citrus* scion varieties have shown marked variation in size and vigor, within varieties, over a fifteen-year period (Mukherjee and Cameron, 1958).

Table 5–5

IDENTITY AND CONDITION OF THIRTY- TO THIRTY-FIVE-YEAR-OLD TETRAPLOID CITRUS TREES OF SEVERAL VARIETIES AT RIVERSIDE, CALIFORNIA, IN 1963

Variety or Selection	Number of Trees*	Condition Relative to Nucellar Diploids†	
		Size	General Vigor
Grapefruit:			
Hall's Silver	4	Smaller	Nearly equal
Imperial	1	Equal	Equal
Royal	2	Nearly equal	Equal
Seedy (white)	3	Smaller	Slightly poorer
Lemon:			
Lisbon	5	Much smaller	Poorer
Orange:			
Paperrind	4		Slightly poorer
Ruby	3		
Seedy sweet	2		
Washington navel	1		Much poorer
Mandarin:			
Dancy	1	Much smaller	Much poorer
King	7	Smaller	
Willowleaf	2	Much smaller	
Satsuma	2	Smaller	Slightly poorer

* Includes repropagations on various rootstocks and some original seedlings. All selections originally obtained as spontaneous nucellar tetraploids.

† Comparisons are based on trees of the same age on the same rootstock.

In most varieties at Riverside (excepting Lisbon lemon and King mandarin), tetraploid fruits have been less elongate than diploid ones, and have shown more basining at the apex (Frost, 1932). Tetraploid fruits are commonly smaller, especially on weak, outer branches; yet with the Lisbon the tetraploid fruits have been larger than those of the diploid and rougher and more irregular in shape. Irregular fruit shape seems characteristic of tetraploids, and the fruits usually have thicker rinds (fig. 5–18) and larger and more prominent oil glands. The juice vesicles are tougher and the yield of juice, on the basis of whole fruit weight, is much lower. Tetraploid fruits tend to color later, but their flavor at maturity seems little affected, and the content of acid and soluble solids is similar to that in diploids. In the high-yielding tetraploid Lisbon, acid content has usually been only slightly lower than in the diploid.

Seed production and overall value.—Seed number is low in some tetraploids studied at Riverside, California, but in the Ruby and Paperrind oranges it has been about equal to that in the respective diploids, and in the Lisbon lemon it has been greater than in the diploid. When seeds are few, many of them are often poorly developed; when they are well developed, they tend to be larger, especially longer, than in the corresponding diploid. The number of embryos per seed tends to be fewer in tetraploids, and thus the average size of embryo in well-developed seeds is larger. There is little evidence as to the proportion of sexual embryos in seeds from selfing. Degeneration of pollen mother cells and irregular chromosome reduction (chap. 4) should result in fewer viable gametes than in corresponding diploids. Embryo abortion may also be higher. The variability in level of seed production among tetraploids suggests that more than one causal mechanism is involved. If comparatively regular chromosome reduction occurs in the Lisbon, this would favor the observed fertility. The large fruit size in this form may be a result of its higher seed number or of a general growth tendency which, in turn, favors seediness. Cameron, Cole, and Nauer, (1960) found a strong correlation between seed number and fruit size in diploid Valencia orange and some other diploid varieties.

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Where normal seed production occurs despite very irregular meiosis, some other factor favorable to fertility is indicated. In general, when genes are heterozygous, autotetraploids produce smaller proportions of pure recessive gametes and fewer progeny homozygous for unfavorable recessive genes than do corresponding diploids.

Tree and fruit characters of *Citrus* autotetraploids show that these forms are not likely to have value for fruit production. They produce poorer fruit and usually lower yields than corresponding diploids. They are valuable in breeding, however, since by crossing with diploids some of them can be used to produce nearly seedless triploid varieties.

Triploids: Origin, Characters, and Promise.—Triploids, like tetraploids, occasionally occur spontaneously among citrus seedlings; they may also be produced almost at will by certain crosses. Triploids occur as sexual seedlings, however, while tetraploids characteristically arise as apogamic ones. Frost, in early breeding studies, identified about twenty triploids and observed other probable ones, for a total of more than 5 per cent of about 1,200 hybrids from diploid parents grown to fruiting age at Riverside, California. Indications of triploidy were also found among zygotic progeny from open pollination. Lapin (1937) reported eighteen triploids and one modified triploid (4.27 per cent triploidy) among 445 hybrids from pollination of *Citrus limon* by eight other species and varieties. He also found one triploid hybrid from *C. medica,* five triploids among 707 seedlings from open pollination of *C. limon* and *C. paradisi,* and five among forty-three seedlings of Shivamikan. He found one hexaploid from open pollination of Ponderosa lemon. *Poncirus trifoliata* as seed parent gave no triploids among 123 hybrids, although 353 nucellar seedlings and 147 other seedlings from open pollination included eighteen tetraploids.

Triploidy indicates that an embryo has been produced by union of a monoploid (1*x*) gamete with one having the diploid (2*x*) chromosome number. In the formation of spontaneous triploids, the doubled number is no doubt usually provided by the egg, since competition among pollen grains should favor the normal 1*x* gametes. From the characters of spontaneous triploids, both Lapin and Frost considered that the seed parent had usually contributed the 2*x* gamete.

The frequent occurrence of tetraploids among nucellar seedlings suggests that the doubling which produces triploids occurs prior to the reduction divisions, but it may also result from failure of reduction. If it occurs before meiosis, modified triploids, with more or less than the exact 3*x* chromosome number, should sometimes occur, unless such unbalance seriously reduces viability.

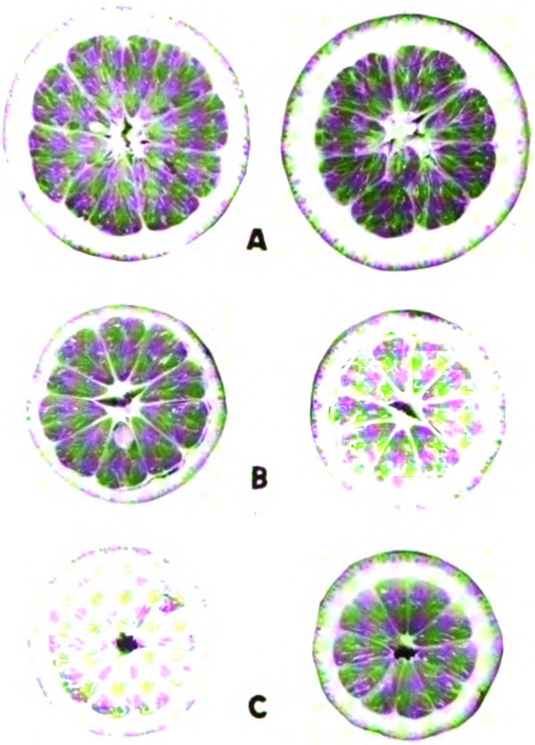

Fig. 5–18. Fruits of *A*, Washington navel orange; *B*, Owari (?) satsuma; and *C*, Lisbon lemon. Diploid fruits in left column, tetraploids in right column. Tetraploids usually have thicker rinds and a lower percentage of juice.

The extra set of chromosomes present in triploids may tend to produce the same general effects, in lesser degree, as do two extra sets in tetraploids, except for the greater sterility expected in triploids. Triploids, however, result from fecundation and show the high genetic variability which characterizes zygotic progeny in citrus. Positive identification of spontaneous triploidy by tree and fruit characters is thus difficult. On the other hand, in controlled crosses where one parent is tetraploid, the occurrence of non-nucellar progeny having thick, rounded leaves and nearly seedless fruit is presumptive of triploidy or near triploidy.

Triploids can be systematically produced by crosses of tetraploids by diploids, although the low incidence of viable seeds is a major obstacle. Longley (1926) determined cytologically that two hybrids of a diploid limequat pollinated by tetraploid *Fortunella hindsii* were triploid. (See also Traub and Robinson, 1937.) Frost (1943) reported that a tetraploid grapefruit and tetraploid Lisbon lemon as seed parents produced triploids in crosses with diploid pollen parents. When the grapefruit was used as pollen parent, very little seed was obtained.

Russo and Torrisi (1953), in two seasons, obtained 307 seeds from 240 fruits of diploid lemons crossed by two selections of a tetraploid lemon (the Doppio). Among sixty-three of these seeds, twenty from the cross of Feminello by tetraploid Doppio produced seven triploids, and forty-three seeds from Monachello by Doppio produced twelve triploids. Tachikawa *et al.* (1961), after producing tetraploids of *Citrus natsudaidai* Hayata and Miyagawa-Wase satsuma by treatment with colchicine, made crosses between these tetraploids and various diploids. In two seasons, diploid Natsudaidai as seed parent crossed by tetraploid Natsudaidai produced thirteen well-developed seeds in twenty-five fruits; the reciprocal cross gave twenty-one seeds in two fruits. Three diploid, monoembryonic seed parents crossed by tetraploid Natsudaidai yielded forty-two seeds from sixty-four fruits. From these crosses and one other, twelve triploids have so far been reported.

At the University of California Citrus Research Center, Riverside, continued crossing to produce triploids has been carried out for several years. Tetraploids have been used as seed parents and as pollen parents; eight tetraploid varieties and some twenty-four diploid ones have been included. Table 5–6 summarizes the types of crosses and the seeds and seed germination obtained. Numbers of developed, but empty seed coats have usually been high when the pollen was tetraploid, regardless of whether the seed parent produced only zygotic or in part nucellar seedlings. When the seed parent was tetraploid (and partly nucellar) and the pollen parent was diploid, greater proportions of normal-appearing seeds resulted. Germination of normal-appearing seeds has been variable in all groups.

Recent cytological studies by J. W. Cameron and R. K. Soost have shown that among the crosses just described, some hybrids which arose from diploid, zygotic seed parents crossed by tetraploid pollen parents are themselves tetraploid.

Table 5–6

SEED DEVELOPMENT AND GERMINATION IN CROSSES BETWEEN TETRAPLOID AND DIPLOID CITRUS VARIETIES AT RIVERSIDE, CALIFORNIA, FROM 1955 TO 1962

			Seeds or Seedcoats Obtained			
Seed Parent	Number of Pollen Varieties	Number of Fruits Obtained	Apparently Well-Developed	Developed But Empty	Total Per Fruit	Seeds Germinated Per Fruit
Diploid, zygotic:	*Tetraploid*					
Temple (tangor?)	4	12	20	133	12.8	1.7
Clementine mandarin	1	21	4	101	5.0	0.1
Wilking mandarin	3	10	49	29	7.8	1.9
Kincy mandarin	2	5	20	22	8.4	3.8
Sukega orangelo	5	81	387	904	15.9	3.9
Pummelo (CRC 2240)	1	7	64	103	23.9	1.3
Diploid, partly zygotic:[*]	*Tetraploid*					
Eureka lemon	1	6	18	51	11.5	2.2
King mandarin	5	23	47	167	9.3	1.5
Minneola tangelo	2	38	95	566	17.4	1.4
Pearl tangelo	1	63	53	989	16.5	0.4
Tetraploid, partly zygotic:	*Diploid*					
Lisbon lemon	1	17	84	35	7.0	2.5
Paperrind orange	5	11	31	18	4.5	0.5
Ruby orange	7	20	65	25	4.5	1.6
Sweet orange (seedy)	8	31	168	24	6.2	3.6
King mandarin	1	1	1	5	6.0	0

[*] Partly zygotic indicates that a seed parent produces some zygotic and some nucellar seedlings.

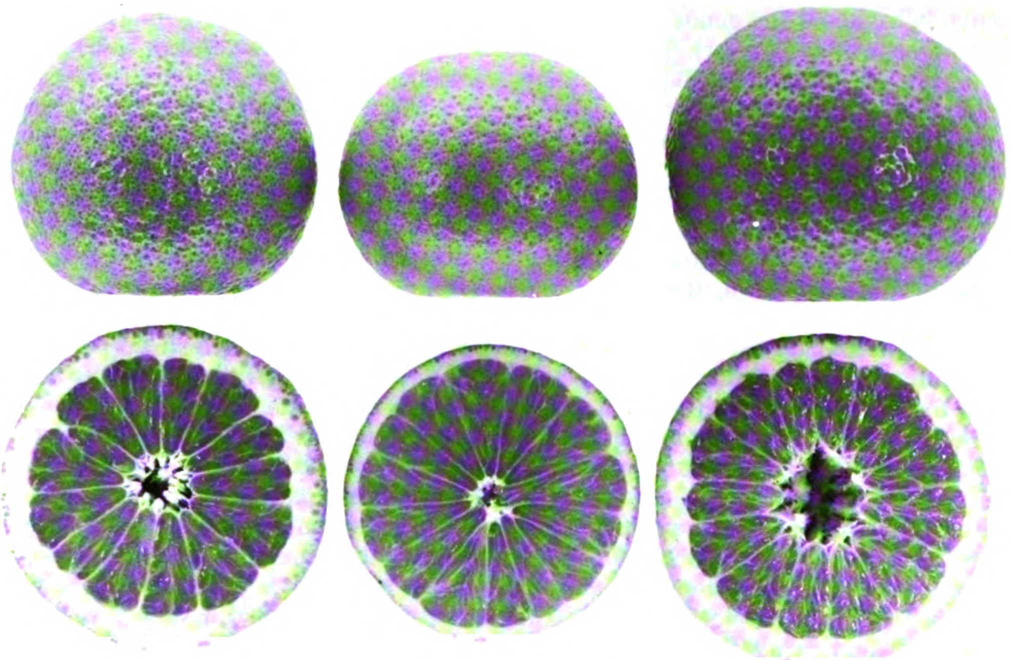

Fig. 5–19. Whole and cut fruits of three different triploid hybrids from the cross, 4*n* seedy white grapefruit × 2*n* Dancy tangerine. Most triploid hybrids are nearly seedless.

This is in agreement with the report of Tachikawa *et al.* (1961).

Since the chromosomes of triploids are present in triplicate before reduction, the gametes should usually receive unbalanced sets, and much sterility is to be expected. In the known citrus triploids studied, the fruits are nearly seedless (fig. 5–19). Some triploids set fruit well, whereas others are very unproductive. Thus, among seven spontaneous triploid hybrids obtained by Frost, long-term yields have been good in four and very poor in three. Among twenty-eight putative triploids from controlled pollination of tetraploid grapefruit by diploid Dancy tangerine and diploid Kinnow mandarin, about one fourth have yielded moderately well. These twenty-eight and a larger number from tetraploid lemon crossed by various diploid pollen parents have all had fruit with very few seeds, averaging from less than one seed up to three or four. Seed numbers in the parent types range from about eight to more than twenty-five.

The production of triploids, by crossing tetraploids with diploids, provides an important method of producing essentially seedless varieties of *Citrus* at will. Seedlessness is of great horticultural importance and is seldom found among diploids except in a few selected varieties, so that it is desirable to test extensively the possibilities of such crosses. It is of interest, also, to determine the characters of hybrids in which one parent has provided two thirds of the chromosomes. The tendency to low fruit yields is a serious practical problem as is the usual low incidence of superior genetic types from crossing.

CITRUS BREEDING

Breeding is the artificial control of reproduction and therefore of inheritance. It involves the selection of parents and usually selection among the resulting progeny. Breeding and genetics are intimately interrelated; each can further the development of the other. Although simple selection for desirable forms, without knowledge or control of the pollen parent, may be considered a form of breeding, controlled cross- or self-pollination is usually basic to a breeding program. Within the last twenty years, "mutation breeding" by the use of irradiation and of chemical mutagens has become an approach of interest with many plants, but it has so far been very little used in citrus.

Problems and Needs in Citrus Breeding

The particular problems associated with citrus breeding are reflected by the genetic and reproductive behavior of the genus (chap. 4 and previous portions of this chapter). The most unusual feature is, of course, nucellar embryony. This mechanism limits, and often essentially precludes, crossing and selfing in many varieties. When first generation progeny are obtained, their degree of nucellar embryony may be greater or less than in their parents, depending upon the cross. In addition, various varieties and hybrids such as the satsuma mandarin, the Washington navel orange, and the Morton citrange, are highly pollen-sterile. Self- and cross-incompatibility are more common than had previously been suspected. On the other hand, great gene diversity, frequent bud variation, and the overall interfertility among *Citrus* species provide a wide range of material for breeding purposes.

Important breeding goals exist in citrus with respect to both scions and rootstocks. Many needs are of long standing, and resemble those in other tree fruits. Vigor and longevity of tree and sufficient amount and regularity of crop are important. In scion varieties, fruit size is a critical factor and many hybrids of otherwise good quality are discarded because the fruit is too small. With varieties to be eaten out-of-hand, seed content is also important. Many of the varieties now grown for fresh fruit in the United States have few seeds, with the notable exception of many tangerine types and certain juice oranges. Seedlessness is difficult to obtain by regular breeding methods, however, since mechanisms related to seed production often affect fruit development. Fruit shape, rind appearance, and flavor are of major importance. Season of ripening, storage life, and adaptability to local environment often determine whether a new variety will be grown. In Japan, the satsuma is a major variety partly because the fruits can be harvested before cold weather begins. In South Africa, storage life is important, since much of the fruit is exported; in the Coachella Valley desert area of California, high foliage density and fruits relatively resistant to sunburn are essential.

Most prominent citrus varieties suffer one or more defects, even in growing areas to which they are well adapted; these defects are often difficult to remedy. The Washington navel orange tends toward low productiveness under many conditions (perhaps as a consequence of its otherwise desirable seedlessness). The Valencia can suffer from small fruit size and granulation of the flesh. Grapefruit, except in a few favored areas, is often rather sour and bitter early in its season.

Certain characteristics being sought in new varieties in the United States have recently been summarized by Cooper (1962). They include new mandarin types suited to various seasons, new sweet oranges, increased cold hardiness in scions and rootstocks, and rootstocks resistant to fungi, nematodes, and virus diseases. In Florida especially, orange varieties combining early maturity, high productivity, and high soluble solids are sought for processing purposes. For use as fresh fruit, oranges attractive in appearance, easy to peel, and high in solids are needed. Seedlessness is of less concern in some areas than in others.

Unusually early-maturing or late-maturing new forms are always of interest, since they may fill a need in a pattern of production or marketing.

In contrast to scion types, successful rootstock varieties must produce many seeds and be highly nucellar. Stock-scion interactions are of critical importance. Physiological reactions and disease reactions are relatively specific in relation to the stionic combinations used, and new hybrids must be evaluated for these reactions.

Breeding Procedures in Citrus

Selection of Parents.—The selection of parents in citrus breeding is governed by a number of considerations. Varieties which are entirely zygotic are to be preferred as seed parents. The pummelos are such a group, and natural crossing and selfing in the past have no doubt given rise to most of the existing varieties. Many known or putative hybrids that are apparently entirely zygotic are now recognized among other citrus groups. They include the Clementine, Kishiu, and Wilking mandarins, the Temple (tangor?), Clement tangelo, Umatilla tangor, and other unnamed crosses. Varieties which can produce a useful proportion of hybrid embryos have been used as parents; among these are the King mandarin, Ruby orange, and Eureka and Lisbon lemons. Highly nucellar varieties have been used, especially if they have certain desirable characteristics. Most of the earlier hybrids which have gained some commercial usage, such as the Orlando and Minneola tangelos and the Kara and Kinnow mandarins, arose from the second and third types of seed parents. However, three recent hybrids, Robinson, Osceola, and Lee (Reece and Gardner, 1959), were produced from a zygotic seed parent, Clementine.

Selfing and genetically narrow crossing are

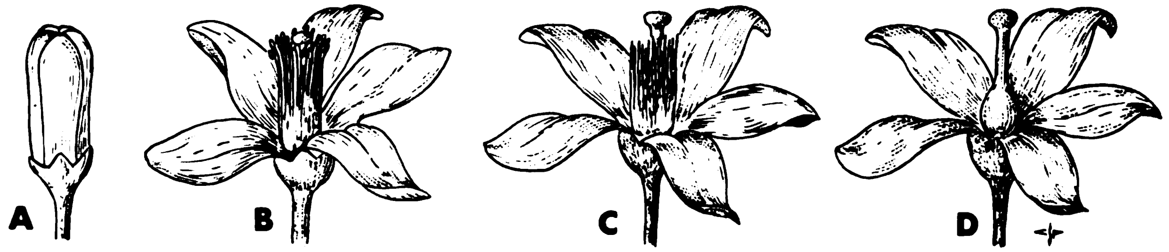

Fig. 5–20. Citrus flower before and after emasculation. *A*, large unopened bud; *B*, complete flower with petals separated; *C*, anthers removed, filaments intact; *D*, anthers and filaments removed.

expected to produce progeny somewhat similar to their parents; however, as previously discussed, these progeny are likely to be weak or otherwise inferior. Selfing of more heterozygous F_1 hybrids may be useful, but it has not yet been adequately investigated. Wider crosses and moderately wide, second-generation intercrosses have so far proved the most promising.

Citrus forms showing characters to be desired in the progeny are commonly used as parents, but there is no assurance that such characters will regularly appear in the hybrids. A quantitative range of phenotypes is the usual pattern in F_1 and to a great extent also in advanced crosses. Unfavorable characters such as bitterness, unpleasant rind or juice oil, or tough segment membranes may appear when the parents show little of these faults. Since hybrids often show many differences from their parents, it is sometimes difficult to classify them under a given parent group, such as orange or grapefruit. An occasional F_1, however, does show very predominantly the characters of one parent; an example is the fruit of the Orlando tangelo (grapefruit × tangerine), which in rind and flesh color, flavor, size, and length of season resembles the tangerine parent much more than the grapefruit.

When a seed parent produces some nucellar embryos, use of a pollen parent with markedly different leaf characters is helpful, since it permits early identification of a part of the hybrids. Many doubtful cases, however, must be carried to fruiting. Seedling tests involving *in vitro* color reactions of bark or leaf tissue (Furr and Reece, 1946) have not been fully dependable.

Techniques for Controlled Pollination.— Controlled pollination in citrus is relatively easy to achieve. When certainty of parentage is required for genetic or taxonomic studies, seed parent and pollen parent flowers should be protected against contaminating pollen. Trees and branches especially likely to set fruit should be used; those which have just borne a heavy crop may not flower, especially in varieties which are alternate bearing. The earlier and larger flowers of a cluster are preferable, but only those with full-sized pistils should be used.

Emasculation is easier and less injurious if the flowers are nearly ready to open (fig. 5–20); flowers with dehiscing anthers must be avoided. Dehiscence usually occurs soon after the petals separate, but in varieties such as the pummelos it often occurs in the closed bud. Several flowers may be emasculated on each selected twig. Setting of fruit is perhaps favored by removing other blooms from a considerable surrounding area. Emasculation is accomplished by gently separating the petals with forceps and pulling off the anthers, avoiding contact with stigma. Forceps may be periodically sterilized in alcohol. Emasculated twigs may be enclosed by flat-bottomed paper bags, tied with twine or pliable wire. To exclude small insects and minimize motion of the bag, cotton pads are often wrapped around the twig at the level of tying. Unemasculated buds must be eliminated from bagged twigs. For selfing, bagging alone is often sufficient, without manipulation of the flowers.

Special storage of pollen is seldom necessary for crosses within the genus *Citrus,* except for long distance transportation, since most varieties have similar and rather long blooming periods. *Fortunella,* however, blooms much later than *Citrus* in many areas, and *Poncirus* may bloom earlier or later, depending on climatic conditions. Kellerman (1915) reported that stored *Citrus* pollen, collected in Florida and packed in Washington, D.C., showed some germination about six weeks later in Japan. Toxopeus (1931) stored mature, unopened buds over a drying agent; he obtained 60 per cent germination after three weeks and produced hybrids after four weeks. Soost and Cameron (1954), using *Poncirus* pollen, obtained hybrids after 20 and 36 days storage of dehisced anthers at 4° C, with and without a drying agent.

Pollination may be carried out immediately after emasculation, or up to several days later, depending upon the condition of the seed parent flowers. Receptivity of the stigma is indicated by an abundant, sticky secretion; unpollinated flowers remain receptive for several days. Flowers to provide pollen are collected just before opening, and the anthers are allowed to dehisce. Pollen may be applied by use of the entire flower (with stigma removed) or dehisced anthers may be placed in a vial and the pollen applied with a brush.

Cross-pollination can be accomplished more rapidly if the twigs are not covered with bags. Furr, Carpenter, and Hewitt, (1963) found that bees seldom visit citrus flowers whose petals have been removed; they emasculate and strip the petals from receptive, unopened flowers, and pollinate immediately. Most later, accidental contamination should thus be avoided.

Artificial Induction of Genetic Changes.—Little has been reported on the use of irradiation or chemicals for the induction of mutations in citrus. Haskins and Moore (1935) treated seed of five species with X-ray. Various seedling abnormalities were observed, including bud fasciation, leaves with split midribs, and a high incidence of albinism. Occasional plants with bi- or trifoliate leaves were noted. These variations were at least partly temporary effects due to injury to cell processes. First-season flowering in grapefruit, which is known to be high as compared with other citrus, seemed to be increased.

Hensz (1960) obtained several thousand plants from irradiated seeds and budwood of grapefruit and of Valencia orange. In this material, the LD_{50} (lethal dose for 50 per cent) for X-rayed seeds fell between 5,000 and 10,000 roentgens; for seeds treated with thermal neutrons, the LD_{50} lay between 6 and 15 hours exposure. With X-rayed budwood it was near 6,000 roentgens. Leaf variegations and distortions were observed. One seedling from Webb Redblush grapefruit had trifoliate leaves. Another (Hensz, unpublished) produced an albino branch.

In 1962, Cameron (unpublished) obtained growing shoots from nearly all of 300 Valencia buds irradiated in a nuclear reactor. The exposures were from 5,800 to 8,300 roentgen equivalents man, derived from a mixture of neutrons and gamma rays. After five years there was little indication of mutation in the resulting plants.

Colchicine was used by Tachikawa, Tanaka, and Hara (1961) to produce tetraploids of Miyagawa Wase satsuma and of Natsudaidai. The latter tetraploid was then used in crosses that produced triploids.

History and Progress of Citrus Breeding

Work of the U.S. Department of Agriculture.—The United States Department of Agriculture has carried on citrus breeding programs for more than seventy years, beginning before 1900. The work may be conveniently divided into three periods.

The earliest period.—The first recorded artificial hybridization of *Citrus* was carried out by Swingle and Webber in Florida in 1893, in relation to disease problems, but a severe freeze in the winter of 1894–95 destroyed most of the seedlings. Impressed by the freeze injury to citrus plantings, Swingle proposed using the cold-resistant trifoliate orange as a parent in crosses to produce hardy hybrids with good fruit. This project was undertaken, together with a program to obtain loose-rinded fruits resembling orange or grapefruit and intermediate varieties.

Seeds from crosses made from 1897 through 1899 were planted in greenhouses in Washington, D.C., and the hybrids obtained were tested in Florida. About 1,780 seedlings were grown, but the number of hybrids was much less since the greater part of the seedlings were nucellar. The principal results were reported in various papers (e.g., Swingle, 1910; Webber and Swingle, 1905; Webber, 1900, 1906). Several important features of citrus genetics, especially the general interfertility of wide crosses and the high variability among F_1 hybrids, were discovered in the course of this work, and some distinct new varieties of horticultural value were produced.

Two tangelos, the Sampson and the Thornton, hybrids between the grapefruit and the Dancy tangerine, at once indicated promise for this type of cross. These varieties have since been grown commercially on a small scale, although they are now seldom planted. The Sampson has also been used as a rootstock because of its vigorous growth, resistance to gummosis, and high de-

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gree of nucellar embryony.

The crossing of the trifoliate orange, *Poncirus trifoliata*, with the sweet orange, *Citrus sinensis*, produced many nucellar seedlings, and some hybrids. These were highly variable, and several produced fruit with certain good characters, but none was satisfactory in flavor. The best of them proved rather sour and all had the disagreeable rind oil of the trifoliate parent, as well as an unpleasant flavor in the oil droplets of the juice vesicles. They were hardy enough, however, to succeed hundreds of miles north of the main Florida citrus region. Several of these citranges, including the Rusk, Morton, Savage, and Cunningham, have been used as rootstocks.

Various other combinations of varieties were used in cross-pollination (Webber, 1900), but none of the resulting hybrids proved commercially valuable. For example, hybrids between orange and lemon were obtained, but they were not especially good either for the uses of oranges or of lemons.

The second period.—Beginning in 1908, W. T. Swingle and associates produced large numbers of hybrids in Florida, from many combinations of varieties and species (Swingle, Robinson, and Savage, 1931; Traub and Robinson, 1937). Several hundred citranges were produced, and citranges were backcrossed with sweet orange, giving citrangors. None were obtained, however, which combined a high degree of hardiness with good orange fruit quality. The most promising citranges appeared to be a partial substitute for the lemon (Swingle and Robinson, 1927).

Some citranges have a defect with respect to hardiness, in that early spring leaf flushes may be injured by frost. Kumquats *(Fortunella)* were therefore also used in crossing because of their exceptional winter dormancy, which extends over a very long period. Hybrids between the kumquat and the trifoliate orange appeared unpromising, but crosses with both citranges and limes were named and released (Swingle and Robinson, 1923). The Thomasville citrangequat is hardy and has little of the unpleasant oil flavor of the citranges. Glen citrangedin, a cross of the Calamondin with the Willits citrange, is likewise hardy, and produces acid fruit similar to that of the Mexican lime, and is free from bitterness (Swingle *et al.*, 1931). Three limequats, the Eustis, Lakeland, and Tavares, were introduced. Their fruits are sometimes classed as limes, but the trees are much hardier.

Another acid hybrid, the Perrine "lemon," was obtained from a cross between the Mexican lime and the Genoa lemon. It appeared hardier than common lemon and was resistant to citrus scab. Because of other disease problems, it is no longer grown (Cooper, Reece, and Furr, 1962).

Because of the good qualities of the Sampson and Thornton tangelos, additional hybrids were produced between the grapefruit and mandarin groups. Several of these were named and described (Swingle, *et al.*, 1931). Two of them, the Orlando and the Minneola, which, interestingly, arose from sister fruits of grapefruit pollinated by Dancy mandarin, have been notably successful. They are considerably different from one another in shape, flavor, and time of maturity. Both are grown in Florida and to a smaller extent in California. Another hybrid of related background is the Wekiwa. It is a backcross of Sampson tangelo to grapefruit, with sweet, pink-fleshed fruit. Other tangelos from this period of crossing have been used in further breeding.

Reciprocal crosses among sweet orange varieties were also attempted. Several hundred of the resulting seedlings were selected as possible hybrids, but many were not carried to fruiting (Cooper, *et al.*, 1962). Since most orange varieties are highly nucellar, it is probable that many of these seedlings were not hybrids.

The recent period.—In 1942, an extensive series of crosses was made at the U.S. Department of Agriculture Horticultural Field Station at Orlando, Florida. The parents included mandarins, tangelos, grapefruit, oranges, and other varieties. Progeny from one cross in particular, Clementine by Orlando, yielded a number of promising hybrids. In a population of 327 seedlings, a wide range of forms occurred, most of them predominantly mandarin in type. Three of these, recently introduced (Reece and Gardner, 1959), are Robinson, Osceola, and Lee. Under Florida conditions, all have large, sweet, early-maturing fruit of orange or red-orange color. Robinson and Osceola resemble tangerines, while Lee has some characters suggesting tangelo or orange. All are earlier than the commonly grown Dancy tangerine. A fourth hybrid, called Nova, was obtained from this same cross (Reece, Hearn, and Gardner, 1964). It resembles the Orlando in size and shape, but is sweeter and earlier-ripening. The Page, a cross of Minneola × Clementine (Reece, Gardner, and Hearn, 1963), is early and has many orange-like characters.

Furr and Reece (1946) in an effort to distinguish between hybrid and nucellar seedlings, applied a color test adapted to leaf extracts to 3,851 seedlings of the crosses referred to above. Where Clementine was seed parent, all seedlings seemed to react as hybrids. These workers then crossed Clementine by the trifoliate orange, so that hybrids could be identified by the dominant trifoliate character. All seedlings produced trifoliate leaves, indicating that nucellar seedlings are apparently not produced by Clementine. With the same crossing procedure, Temple (tangor?) also yielded only zygotic seedlings.

Beginning in 1948, the U.S. Department of Agriculture undertook an expanded program of citrus breeding, centered at its field stations at Indio, California, and Orlando, Florida. Goals and accomplishments of this program are described below.

Breeding studies reported by Furr *et al.* (1963) involved 155 different crosses made to produce edible fruit types. Thousands of seedlings were obtained and grown to fruiting in close field plantings. Mandarins, sweet oranges, tangors, and tangelos were the parents most often used. The size of progeny families varied widely; as expected, the degree of nucellar embryony of the seed parents affected the proportions of hybrids obtained. Table 5–7 shows the principal seed parents used and the numbers of hybrids selected for second test. Clementine and Temple, both having many good characters and producing only zygotic seedlings, were most often used as seed parents and yielded most of the second-test selections. The bulk of these had mandarin or tangelo as the pollen parent. Umatilla, a zygotic tangor, also produced some promising hybrids. Some hybrids of good flavor were obtained from Kao Pan pummelo crossed by Minneola and Orlando. This is the same pummelo clone which produced a favorable hybrid, the Chandler, in other breeding work (Cameron and Soost, 1961). Several thousand flowers of the highly female-sterile Washington navel orange, pollinated by four other varieties, yielded no hybrids.

Red grapefruits as seed parents were highly nucellar. However, a few hybrids between them, and between them and red-fleshed pummelos, all had some color in the flesh. One hybrid of red grapefruit × Foster pink grapefruit had deep red flesh, and two hybrids of this same seed parent × Thompson pink showed slight pink color. The regular presence of pigment in these hybrids suggests that the color may be rather simply inherited.

Three tangerine-type hybrids obtained in these studies were described by Furr (1964). They are Fairchild, Fremont, and Fortune, crosses of

Table 5–7

PRINCIPAL SEED PARENTS YIELDING HYBRIDS SELECTED FOR SECOND TEST AS SCION VARIETIES IN U. S. DEPARTMENT OF AGRICULTURE BREEDING STUDIES

Seed Parent		Pollen Parent	Second-Test Selections	
Group or Variety	Number of Varieties	Number of Varieties	Number of Different Crosses Represented	Total Selections
Citradia	1	1	1	1
Red grapefruit	2	6	1	1
Lemon	4	4	2	2
Mandarin:				
Clementine	1	47	21	94
King	1	8	5	13
Honey	1	6	2	12
Pummelo:				
Kao Pan	1	3	2	5
Other	3	....	0	0
Tangelo:				
Clement	1	12	1	1
Tangor:				
Temple•	1	40	16	31
Umatilla	1	7	5	16

Source: After Furr *et al.*, 1963.

• Parentage of the Temple is uncertain.

the Clementine mandarin by Orlando tangelo, Ponkan mandarin, and Dancy mandarin, respectively. All seem as coldhardy as the Clementine or Dancy and at the same time are tolerant of heat. Under hot desert conditions, Fairchild is the earliest ripening, Fremont is second, and Fortune is the latest.

Resistance to chloride injury was studied in some 500 hybrid seedlings by the U.S. Department of Agriculture (Furr *et al.*, 1963). In several crosses, at least one parent such as Rangpur lime or Shekwasha mandarin was chosen as somewhat salt tolerant. The other parent was sometimes a cold-resistant variety, such as trifoliate orange or sour orange. Original F_1 plants were subjected to water high in chlorides, visual symptoms of injury were rated, and the chloride content of dried leaves was determined. A wide range of injury and of chloride content occurred within crosses, but there was a tendency for more progeny plants to show tolerance when both parents were tolerant. Thus, among fifty-two seedlings of Rangpur lime × Cleopatra mandarin, thirty had less than 1 per cent leaf chloride and showed rather little plant injury. A group of the most tolerant selections, including one each from 18 crosses, showed relatively low leaf chloride (from 0.11 to 2.22 per cent) and low defoliation (from 0 to 25 per cent), although the two characteristics were not always closely correlated.

Testing of varieties and hybrids for tolerance to *Phytophthora* root rot was begun in 1956 as part of a program to obtain better rootstocks (Carpenter and Furr, 1962: Furr *et al.*, 1963). Seedlings were exposed to water suspensions of *P. parasitica* Dastur and the plants were then held in growing beds for selection of survivors. Water-culture inoculation is a severe treatment, and in most seedling lots only a low percentage of plants survived.

Among open-pollinated seedlings of existing rootstock varieties there was evidence of tolerance from widely varying sources, although reinoculation did not always give the same results. Carrizo and Yuma citranges, some pummelos, citrangors, and citrumelos, and some selections of trifoliate orange were among the more tolerant. *Severinia* and a *Microcitrus* hybrid were highly tolerant. In most of these tests the seedlings were no doubt nucellar, and represented the parent genotype, but with the pummelos, and apparently with some *Severinia* selections, they were genetic segregants.

New hybrid seedling populations showed wide differences in survival. Backcrosses of Clementine hybrids to Clementine showed poor survival. Suen Kat and Sunki mandarins, certain pummelos, and the Shekwasha, crossed by trifoliate and by certain citranges, often gave high tolerance; in these progenies, hybrid seedlings were identified either by contrast to the seed parent or by the trifoliate leaf conditioned by the pollen parent.

Other recent hybrids were grown to fruiting, and their open-pollinated seedlings were evaluated by water-culture inoculation. Again, hybrids involving trifoliate orange were notable for seedling survival. A few seedlings from hybrids of Clementine × pummelo showed tolerance, but many were susceptible. These seedlings were presumably all sexual, while those involving trifoliate appear to have been both sexual and asexual. As with salt tolerance, inheritance of *Phytophthora* tolerance appears quantitative. Resistance to trunk and bud-union infection in the field is not always closely related to root tolerance after water-culture inoculation, nor is tolerance to *P. parasitica* the same as to *P. citrophthora* (Sm. & Sm.) Leonian.

Breeding for cold hardiness is being emphasized in the U.S. Department of Agriculture program (Furr and Armstrong, 1959; Furr *et al.*, 1963). This goal may be approached from at least three standpoints: hardiness of the scion, hardiness of rootstock, and reduction of fruit loss by production of early-ripening fruit which escapes freezes. Crosses being studied often include one of these approaches together with efforts to incorporate other favorable characters. Trifoliate orange may offer a source of long range improvement in scion hardiness, but it may be that only advanced crosses can provide acceptable fruit quality. The satsuma and Changsha mandarins, the Meyer lemon, the kumquat, and existing kumquat hybrids are being used as more direct sources of hardiness.

Varieties with early-maturing fruits, being used as parents, include the Clementine, Honey, and satsuma mandarins, Hamlin orange, and Pearl and Orlando tangelos. Preliminary results indicate that Clementine × Hamlin, and Clementine × satsuma produce some early hybrids. Crosses for cold hardiness in rootstocks have involved the trifoliate orange, the sour orange, and several citranges. Segregation for cold hardiness was observed among hybrid seedlings of Rangpur lime

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× Brazilian sour orange.

Work of the University of California Citrus Research Center.—Citrus breeding studies were begun at the University of California Citrus Research Center in Riverside, California, soon after its establishment. These studies may be described under two periods.

The first period.—From pollinations made between 1914 and 1916, H. B. Frost obtained nearly 5,000 seedlings, including about 500 from selfing and the rest from cross pollination (Frost, 1926*b*). Most of the first group were asexual; about one fourth of the second group were hybrids, belonging mainly to the tangelo, tangor, tangemon, lemelo, oramon, and mandarin groups. The parent varieties most often represented were King, Willowleaf, and satsuma mandarins, Dancy tangerine, Imperial grapefruit, Eureka and Lisbon lemons, and Ruby, Valencia, and Maltese Oval oranges. The King proved to be an outstanding parent for the production of good-flavored hybrids, especially in crosses with other mandarin types. Three hybrids, the Kara (satsuma × King), Kinnow (King × Willowleaf), and Wilking (King × Willowleaf), all obtained from rather small populations, were introduced (Frost, 1935); they are now being grown commercially to a limited extent. All have high soluble solids and good flavor. Kinnow and Wilking have unusually long seasons of use, but are strongly alternate in bearing. Wilking, especially, can have small fruit when crops are heavy.

The Pearl tangelo (Imperial × Willowleaf) was also produced from these early crosses (Frost, 1940). It is early-ripening, with a mild, sweet flavor, although seedy and sometimes small in size. An early-season orange, the Trovita, obtained from a nucellar seedling presumably from a Washington navel fruit, was described (Frost, 1935). Under the climatic conditions at Riverside, it is the earliest, low-seeded juice orange yet tested, but it often has objectionably small fruit.

Two additional hybrids, the Frua tangerine (King × Dancy) and the Dweet tangor (Maltese Oval × Dancy), were described later (Frost and Cameron, 1951). Frua trees, however, have shown low vigor; fruit of the Dweet is good-sized, juicy, and of good flavor, but too fragile for eating out-of-hand.

Several other hybrids from this early work were given tentative names, but were not officially introduced because of defects. One of these, called Honey (King × Willowleaf) is unusually early but commonly too small. Others include Mency (Maltese Oval orange × Dancy) and Ruddy (King × Maltese Oval). Both have several good characters, including heavy yielding ability. All of these hybrids have been used in further breeding. Hybrids of lemons with mandarins and with oranges were obtained, but as with similar early crosses made by the U.S. Department of Agriculture, they have not found commercial use.

The later period.—Between 1928 and 1945 several thousand seedlings, mainly hybrids, were obtained from three principal groups of crosses to produce scion varieties. The parentages were: (1) mandarins and mandarin hybrids such as tangors and tangelos; (2) tetraploids crossed by diploids, which yielded the first triploids reported from such planned crosses; and (3) a series of pummelo varieties pollinated by mandarin types, grapefruit, and other pummelos.

Within the first group, King, Dancy and Willowleaf again indicated value in producing hybrids of good flavor; Clementine was also promising in this respect. Among recent hybrids used as parents, Wilking, Dweet, Mency, and Honey proved useful. Wilking has produced several hybrids with deep orange flesh color, oblate shape, and high flavor. A few crosses of Temple by Frua have combined attractive, reddish rind color with relatively early maturity, but poor flavors have often occurred when Temple was a parent. Two mandarin hybrids obtained from crosses of the 1928 to 1945 period were recently introduced (Cameron, Soost, and Frost, 1965). They are Encore (King × Willowleaf) and Pixie (an open pollination from a cross of King × Dancy). Both have unusually late seasons of use, and Encore is especially good until late summer under inland southern California conditions. Pixie, a smaller fruit, is almost completely seedless.

Triploids were obtained from crosses of tetraploid Lisbon lemon and tetraploid seedy grapefruit as seed parents; the pollen parents were Kinnow, Dancy, and certain lemon hybrids. Triploidy or near triploidy was verified by chromosome counts in some of these plants (Frost and M. M. Lesley, unpublished; see also under Triploidy, p. 355).

From the crosses with pummelos, about 800 hybrids were grown to fruiting. The pummelo seed parents included ten selections, ranging from several with rather high acidity to one completely acidless form. Since pummelos ripen several months earlier than grapefruit at Riverside, one aim of these crosses was to produce large fruits

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that might be used like early grapefruits. Fruits of good size were often obtained, although they were usually seedy, and many were bitter or of poor flavor. Most of those with an acid pummelo as a parent were highly acid, but mild, early-ripening hybrids were obtained from crosses with the acidless form (Soost and Cameron, 1961*a*; see also p. 337). A cross of this form with a more acid pummelo produced the Chandler, which is pink-fleshed, early, and non-bitter (Cameron and Soost, 1961). Unusually early, juicy hybrids were obtained from the acidless pummelo crossed by Frua.

Secondary testing of certain hybrids from the crosses discussed above is still under way. Among the most interesting ones are those with unusual seasons of ripening. One hybrid of Clementine × Honey is earlier than either parent. Several hybrids of Wilking × Dweet combine large size, firmness, and good color. A King × Dancy hybrid gives high yields in the hot desert.

Since 1947, the Citrus Research Center breeding program has included several goals. There have been additional crosses to produce orange hybrids and mandarin hybrids. Narrow crosses between Lisbon and Eureka lemon selections are under study to determine whether vigorous hybrids, which maintain typical lemon characters, can be obtained from these parents. Expansion of crossing to produce triploids from several parent varieties has been carried out (see p. 356). Because of the need for vigorous, virus-free Washington navel orange sources, several new nucellar seedling selections were originated from a series of seed parents in 1949. Hybrids for possible new rootstocks, involving trifoliate orange, Clementine mandarin, Ruby orange, *Citrus ichangensis* Swingle, *Citrus taiwanica* Tan. & Shimada, and other parents, have been produced. The evidence that a very low-acid pummelo can contribute to low acidity, and therefore to earliness in its hybrids (see above) has led to advanced crosses of the present F_1 plants with mandarins and with grapefruit. Crosses of an acidless orange with mandarin types and with other oranges have been made. The early-maturing grapefruit hybrid, Sukega, which is entirely zygotic, has been used in several groups of crosses.

Work of the University of Florida Citrus Experiment Station.—The University of Florida Citrus Experiment Station undertook limited citrus breeding in 1924, with emphasis on the production of acid varieties (Traub and Robinson, 1937). Recently, new studies have been initiated (A. P. Pieringer, unpublished). Nucellar seedling selections from commercial varieties are being established from monofoliate seedlings after pollination with the trifoliate orange. Early-maturing, non-bitter grapefruit varieties are being sought in a cooperative breeding program with the U. S. Department of Agriculture. Basic studies on means of identification of species and hybrids are under way.

Studies in countries other than the United States.—Citrus breeding programs were under way in several areas of the world by about 1930, but World War II caused the interruption or abandonment of many of them. In Java, Toxopeus (1931, 1933, 1936) conducted hybridization and related studies, with the primary aim of obtaining better rootstock varieties.

In the Philippine Islands, Torres (1932, 1936) attempted to produce superior scion varieties resistant to diseases and insects. He reported weak hybrids from certain mandarin crosses and from crosses of Calamondin with mandarins.

Large-scale crossing was carried on in the Transcaucasian region of Russia during the 1930's, with the main objective of producing hardy and early-ripening varieties (Luss, 1935; Sukhenko, 1936). Considerable evidence obtained by Lapin (1937) on polyploidy and on nucellar embryony has already been cited.

Frequent reports on citrus breeding and selection have appeared in Russian journals since World War II. Various hybrids have apparently been obtained, but interpretation of methods and results is difficult. Kapcinelj (1948) mentioned a hybrid orange, called Pervenec. Zorin (1949) re-emphasized the importance of hardiness; he stated that several hardy hybrids had been obtained from Kin Kana crossed by Unshu (Unshiu) and that Unshu × Siva Mikan had produced a hardy mandarin. He referred to a combined method of vegetative and sexual hybridization. Later, Zorin (1959) stated that hybrids of three parents had been obtained by this method. Mamporija (1960) described a vegetative hybrid (synthetic chimera?) which arose at the junction of Unshu budded on trifoliate. He stated that neither the sexual nor the nucellar seedling progeny of this plant segregated for new characters, but that they were tetraploid, with thirty-six chromosomes.

Programs of citrus improvement in Japan were long centered on selection among satsuma variants, but in recent years new breeding programs have been initiated. At the Horticultural

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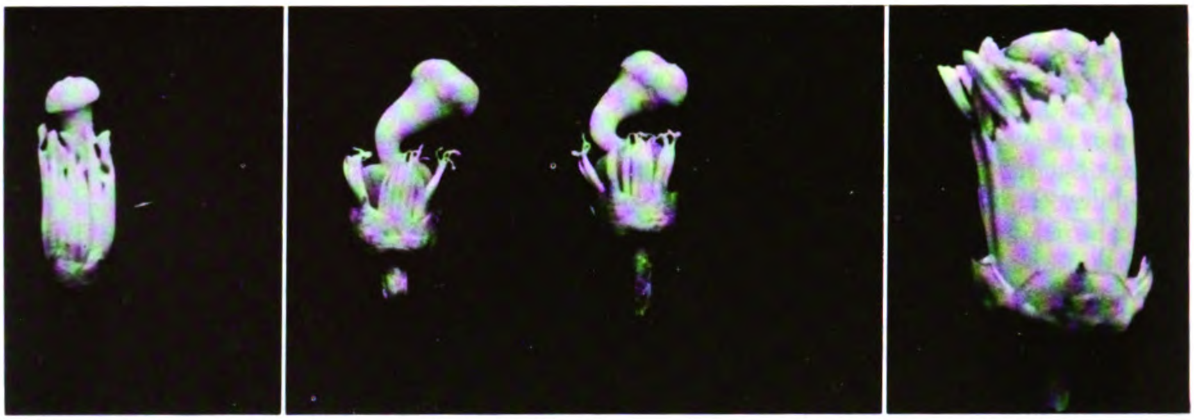

Fig. 5–21. Flowers of satsuma mandarin (left), Hirado pummelo (right), and a hybrid between them (two center flowers) showing aborted anthers of the hybrid. Petals have been removed. (From Iwamasa, 1966.)

Research Station at Okitsu, the satsuma, Washington navel, and certain seedy varieties have been crossed with each other and with *Poncirus* (Nishiura and Iwasaki, 1963). Bud pollination was used to increase seed production in part of these crosses (Iwasaki, Ohata, and Nishiura, 1954); some 8,000 seedlings, including nucellar ones, are currently being studied. Marked differences in parthenocarpy and in degree of seediness were observed among seed parents after cross-pollination. Among a few hybrids obtained with satsuma, Iwamasa (1966) found segregation for plants with undeveloped anthers in a ratio suggesting single gene inheritance (see also Nishiura, 1964). Twenty hybrids, involving nine pollen parents, included twelve with normal-appearing anthers and eight with undeveloped ones. Among the twelve, some produced very little viable pollen, as does satsuma itself. Figure 5-21 shows flowers of a hybrid with undeveloped anthers, contrasted with flowers of its parents.

Segregation for male sterility was also observed by Iwamasa among forty-six hybrids of satsuma crossed by trifoliate orange. In these plants, anthers were developed, but in twenty-six of the forty-six the pollen mother cells degenerated and no mature pollen grains were present.

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CHAPTER 6

Plant-Growth Regulators

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LIVING ORGANISMS are complex structural and biochemical entities. Why a particular species differs from others and how a complex organism arises from a single cell is not clearly understood. It is clear that a high degree of organization of growth processes must exist to account for the orderly development of plants. A few organic compounds that influence certain biochemical and physiological reactions have been isolated from the wide array of organic and inorganic compounds found in plants. A growing body of evidence suggests such compounds are of general occurrence in plants, active at exceedingly low concentrations, and under genetic control. These compounds, which act as chemical directors of growth processes, are known as plant-growth substances or plant-growth regulators.

Although many early workers suggested the existence of chemical control mechanisms in plants, it was not until the twentieth century that observations made by Darwin (1896) led to the isolation of a chemical messenger by F. W. Went (1928). A detailed historical account of plant-regulator research since Darwin's time may be obtained from Skoog (1951), Tukey (1954), Audus (1959), Leopold (1964), and van Overbeek (1966). In recent years, considerable research has been devoted to both naturally occurring and synthetically produced plant-growth substances. Numerous compounds have been investigated, and significant progress has been made in both fundamental and applied research. These compounds, already important in agriculture, are destined to become increasingly valuable as more knowledge of their physiological influence is acquired.

Several naturally occurring plant-growth substances have been isolated from citrus tissues. Auxins were the first endogenous substances to receive attention. Gustafson (1939) found that extracts from flower bud ovaries of parthenocarpic varieties of oranges, lemons, and grapes contained higher auxin levels than ovaries from corresponding varieties that do not produce fruits parthenocarpically. Cameron, Cole, and Nauer (1960) reported a positive correlation between seed number and fruit size for the Valencia orange and trends toward positive correlations in four other citrus varieties. This and other similar evidence led to speculation that seeds directly or indirectly stimulate fruit growth through the action of plant-growth regulators. Monselise (1945) obtained auxin from the growing shoots of Shamouti orange. Stowe, Thimann, and Kefford (1956) reported that orange blossoms contain indole, indoleacetic acid (IAA), and an unknown growth inhibitor. Khalifah, Lewis, and Coggins (1963) found evidence for an auxin in young citrus fruits that does not appear to be an indole. This new auxin also has been isolated in fruits of date, fig, peach, and Natal plum, but no IAA was found in these fruits (Lewis, Khalifah, and Coggins, 1965). Fruits of guava and strawberry contained IAA but not the new auxin. This recent research raises the question of whether the new auxin and IAA have been confused with each other in the past.

Kawarada and Sumiki (1959) found gibberellin A_1 in the elongated water sprouts of *Citrus unshiu* Marc. Khalifah *et al.* (1965) reported evidence for the occurrence of three different gibberellin-like substances in orange and lemon fruits. Two of these compounds appear to be gibberellins A_1 and A_9. Growth-stimulating activity also has been reported from lemon and orange oil and is believed to be due in part to the presence of lauric, linolenic, and linoleic acids (Haagen-Smit and Viglierchio, 1955). Germination- and growth-inhibiting coumarins have been isolated from citrus tissues (Khastagir, 1947).

In the citrus industry, plant-growth substances have proven effective in stimulating a number of desired responses, such as decreasing fruit drop losses, increasing fruit size, improving fruit set, and delaying fruit maturity. Differences in varieties and cultural practices make almost any physiological response useful somewhere in the industry, but to be successful a response must be dependable and reasonably free of undesirable effects. This chapter discusses many of the responses that have a history of practical success and others whose applications are in the preliminary to advanced stages. Plant-regulator research in the future can be expected to discover many more new uses for plant-growth substances.

FRUIT ABSCISSION

Citrus fruits have no well-defined physiological maturity stage. Harvest of oranges and grapefruit in California begins when the fruits are mature enough to be eaten and usually ends four to six months later. Abscission causes serious fruit loss, especially during the latter part of the harvest season.

The first reported citrus research on the use of plant-growth regulators to decrease preharvest abscission came from Florida (Gardner, 1941). Naphthaleneacetamide applied to Pineapple oranges early in the harvest season at a concentration of 100 parts per million (ppm) reduced fruit drop for twelve weeks or longer. A concentration of 10 ppm was ineffective, and even 100 ppm was not effective if applied after preharvest abscission had become a problem. The use of naphthaleneacetamide has not developed into a commercial practice.

In California, Stewart and Klotz (1947) applied 2,4-dichlorophenoxyacetic acid (2,4-D) to Valencia orange trees after the start of preharvest fruit abscission and obtained significant decreases in fruit losses. The literature since 1947 contains numerous reports of mature fruit abscission being reduced by 2,4-D and related compounds. Studies in California indicated that 2,4-D is also effective on navel oranges (Stewart, Klotz, and Hield, 1947), grapefruit (Stewart and Parker, 1947), and lemons (Stewart and Hield, 1950). Other reports could be cited, but the important point is that 2,4-D is being used successfully in California to reduce preharvest fruit abscission (table 6–1). Reports indicate that the same response generally has been obtained in Australia (McAlpin and Merrett, 1949), South Africa (DeVilliers, 1955), Japan (Iwasaki, Nishiura, and Shichijo, 1956), China (Hwang, 1958), and India (Randhawa, Sharma, and Jain, 1961). Results in Florida have been less successful than in California, but 2,4-D has proven effective on Pineapple seedling orange and Temple orange (Gardner, 1951). When applied to other varieties of citrus in Florida, 2,4-D has been ineffective.

Often it is desirable to apply 2,4-D with nutritional or pesticidal sprays. In general, 2,4-D is compatible with all of these sprays in common use in California (Hield and Erickson, 1962). However, Halse (1954) reported that Bordeaux mixture impaired the effectiveness of 2,4-D in preventing fruit abscission. When 2,4-D esters are combined with insecticidal oils, the effectiveness of 2,4-D is increased and the dosage of the regulator must be decreased to avoid plant injury. Fall applications of whitewash are frequent in the San Joaquin Valley of California as an insecticidal treatment. When 2,4-D is incorporated in whitewash, the concentration of the plant-growth regulator must be increased to obtain satisfactory control of abscission (Hield and Erickson, 1962).

Two other compounds, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP) have also proven effective against abscission. In some instances, 2,4,5-T is more effective than 2,4-D (Stewart and Hield, 1950). After Sites (1954) reported that preharvest abscission of Pineapple orange in Florida was reduced by 2,4,5-TP, the compound was tried in California by the junior author in the hope that leaf malformations, which sometimes result from 2,4-D, would not occur. Under California conditions, higher concentrations of 2,4,5-TP proved necessary to obtain abscission control. Once the concentration was high enough to provide control comparable to that obtained with 2,4-D, leaf malformations resulted.

Most leaf distortions can be avoided by ap-

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Table 6–1

THE INFLUENCE OF 2,4-D (ISOPROPYL ESTER FORMULATION) ON PREHARVEST FRUIT ABSCISSION IN CALIFORNIA

				Relative Number of Fruits Dropped per Tree Between Dates Indicated		
Kind of Citrus	California Location	Date Sprayed	Date Counted	Untreated Control	2,4-D (8 Ppm)	2,4-D (16 Ppm)
Navel orange	Arlington	Dec. 16, 1954	Mar. 23, 1955	168	....	81
	Highgrove	Dec. 29, 1954	Mar. 9, 1955	79	....	41
	Piru	Dec. 16, 1954	Apr. 17, 1955	136	....	74
	Piru	Dec. 16, 1954	Mar. 9, 1955	81	36	....
	Redlands	Dec. 17, 1954	Mar. 9, 1955	69	....	25
Valencia orange	Fullerton	June 10, 1947	Oct. 21, 1947	47	32	....
	Santa Ana	June 12, 1947	Aug. 16, 1947	77	36	25
	Tustin	June 11, 1947	Sept. 14, 1947	313	232	270
Lemon	Corona	Mar. 15, 1954	May 25, 1954	100	....	39
	Ventura	Apr. 1, 1948	July 14, 1948	73	61 N.S.*	....
Grapefruit	Brea	May 28, 1950	July 11, 1950	57	19	....
	Indio	Feb. 7, 1955	June 15, 1955	7	2 N.S.	....
	Riverside	June 8, 1959	Aug. 21, 1959	93	....	43

Sources: All data from authors' files, with the exception of Valencia orange data (Stewart, Hield, and Brannaman, 1952) and Ventura lemon data (Stewart and Hield, 1950).

* N.S. indicates that differences between the means are not significant at the 0.05 level of probability. All other comparisons are significant at the 0.05 level of probability.

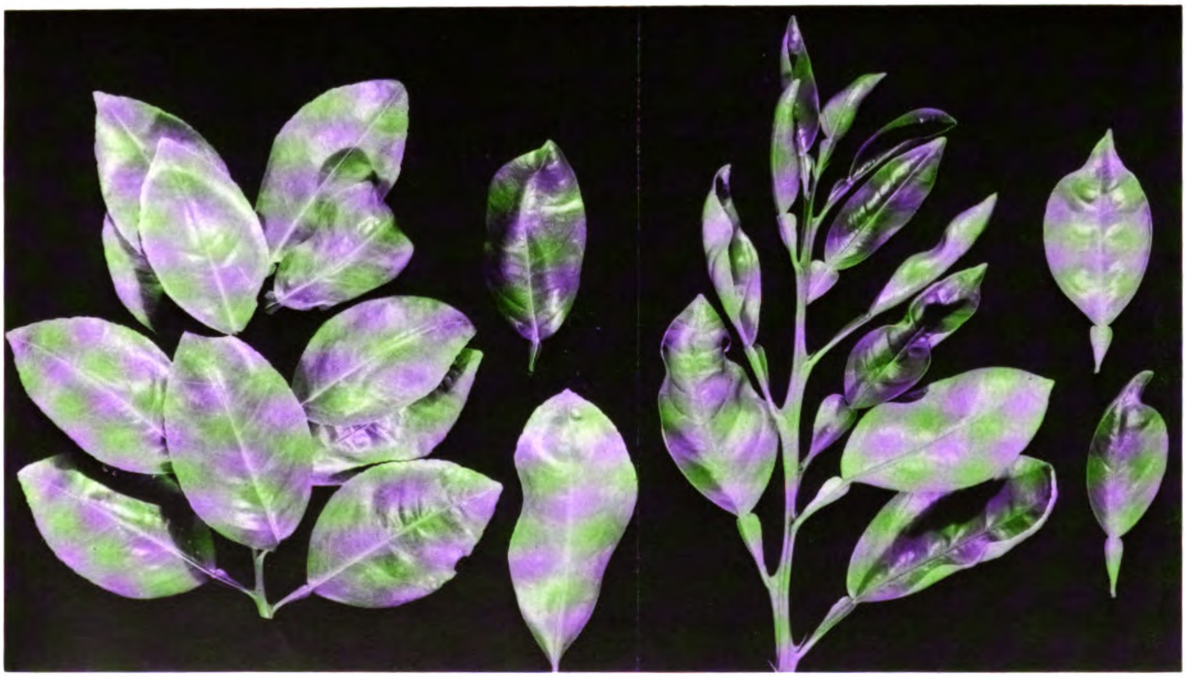

Fig. 6–1. Leaf distortions caused by plant-regulator concentrations of 2,4-D. Left, after application of 2,4-D to young, soft grapefruit leaves. Right, three weeks after application of 2,4-D to grapefruit trees when vegetative buds were swelling. Neither response is serious unless a large number of shoots are involved.

plying regulator compounds when young growing leaves are not present. Typical leaf distortions caused by 2,4-D are shown in figure 6–1. In general, the high volatile ester formulations, such as isopropyl ester, have proven less injurious than low volatile ester formulations. Since 2,4-D applied to green-colored oranges delays the loss of green rind pigments, application of the compound usually is avoided in groves that will be harvested early in the season.

In addition to its effectiveness in control of preharvest abscission, 2,4-D reduces the amount of fruit stem dieback that occurs late in the harvest season (Stewart *et al.*, 1947; Hield and Erickson, 1962). This response, probably caused by the same physiological mechanism that delays abscission, results in a more turgid fruit.

Pesticide oil sprays occasionally enhance leaf and fruit drop. Oil-induced abscission can be reduced by including 2,4-D in the spray mixture (Stewart and Ebeling, 1946; Stewart, Riehl, and Erickson, 1952). Day and Erickson (1954) reported that 2,4-D, 2,4,5-T, and 2,3,4-trichlorophenoxyacetic acid are about equally effective in preventing ethylene-induced abscission of lemon leaves. Moderate control was obtained from 2,3,5-trichlorophenoxyacetic acid. Since the latter two compounds are not very active with respect to leaf malformation, it was suggested that they may be used for abscission control on plants which cannot tolerate 2,4-D and 2,4,5-T.

In both California and Florida, a vigorous research effort is being devoted to a search for compounds that will promote citrus fruit abscission as an aid to mechanical harvesting. Hendershott (1964) obtained evidence that inhibitors of sulphydryl enzymes are more effective in inducing abscission layer formation than those which inhibit dehydrogenase systems or act as uncoupling agents. Wilson (1966) demonstrated that abscission stimulated by iodoacetic acid cannot be morphologically distinguished from normal abscission. The physiological mechanism by which iodoacetic acid produces abscission is not clear. Cooper and Henry (1967) presented a preliminary report showing that field sprays of ascorbic acid can cause a loosening of mature citrus fruit without appreciable drop of leaves or small fruit. This treatment may have limited commercial application, since it causes pitting on the rind of mature fruit.

FRUIT SIZE

When small citrus fruits are treated with high concentrations of 2,4-D, many of them become misshapen (Stewart and Klotz, 1947). Treated navel oranges may develop thick rinds and protruding navels or become cylindrical in shape. Small seedlike structures, mainly enlarged seed coats, develop in navel oranges if high concentrations are applied to small fruits. In addition to this response in the seedless navel orange, similar morphological aberrations have resulted from 2,4-D applications to Valencia orange fruits. Applications of 2,4-D to grapefruit trees have resulted in cylindrical fruits with navel structures, thick, coarse rinds with excessively large oil glands, and fruits with dry, hard juice vesicles (Stewart and Parker, 1947). High concentrations of 2,4,5-T have caused lemon fruits to develop coarse rinds (Erickson and Brannaman, 1950*a*). The same type of response occurs from heavy applications of 2,4-D.

The desirable response from the application of 2,4-D to young fruits is an increase in fruit size. When appropriate concentrations are used (Hield and Erickson, 1962), undesirable side effects cease to occur, with the exception of moderate increases in rind thickness and roughness. The increase in fruit size can be used to economic advantage when a size problem exists if the orchard in question does not normally produce fruits with thick, coarse rinds. A size increase can be obtained in California with navel orange, Valencia orange, grapefruit, lemon, and lime. Some typical results after 2,4-D treatment are shown in table 6–2. Experience with limes is limited (Erickson and Brannaman, 1950*b*), but small fruits are rarely a problem in lime culture. A better fruit size response is obtained with 2,4,5-T than with 2,4-D on lemons and grapefruit (Erickson and Haas, 1956; Hield and Stewart, 1956).

Application of 2,4-D and 2,4,5-T regulators to small fruits causes a delay in loss of green rind pigments, which may indicate that treated fruits are physiologically younger than those from unsprayed trees. This apparent delay in maturation may account for some of the size increase as well as reduced abscission of mature fruits. Additional factors that may contribute to increased size are a thicker fruit stem in proportion to fruit diameter, stimulated growth rate of certain tissues, and a thinning effect or reduced numbers of fruits per tree (Stewart, Klotz, and Hield, 1951; Stewart *et al.*, 1952). The effectiveness of 2,4-D as a "sizing" spray is related to stage of fruit growth. As

Table 6–2

THE INFLUENCE OF 2,4-D (ISOPROPYL ESTER FORMULATION) ON SIZE OF CITRUS FRUITS

Kind of Citrus	California Location	Date Sprayed	Date Measured	Average Fruit Diameter (Mm) Untreated Control	2,4-D (8 Ppm)	2,4-D (16 Ppm)
Navel orange	Corona	June 14, 1955	Jan. 23, 1956	65.3	....	68.1
	Lindsay	June 5, 1956	Nov. 13, 1956	60.0	....	65.7
	Lindsay	June 5, 1956	Nov. 13, 1956	65.1	69.6	69.8
	Porterville	June 4, 1956	Nov. 13, 1956	63.6	....	67.6
Valencia orange	Claremont	June 11, 1955	Mar. 27, 1956	54.6	58.0	....
	Santa Paula	June 14, 1955	Mar. 1, 1956	58.7	62.0	....
	Tustin	June 2, 1954	Apr. 20, 1955	61.2	....	63.6
	Tustin	June 3, 1954	Apr. 20, 1955	56.9	....	58.8
Grapefruit	Fillmore	June 15, 1955	Feb. 29, 1956	81.4	83.2 N.S.*	....
	Highgrove	June 13, 1955	Apr. 17, 1956	83.0	82.3 N.S.	....
	Highlands	June 15, 1955	Jan. 24, 1956	70.8	74.9	....
	Indio	May 26, 1955	Nov. 14, 1955	82.5	86.3	....
	Riverside	June 8, 1959	June 20, 1960	81.0	84.9 N.S.	....

* N.S. indicates that differences between the means are not significant at the 0.05 level of probability. All other comparisons are significant at the 0.05 level of probability.

fruits become larger, they are less responsive to 2,4-D. It should be stressed that 2,4-D does not replace the need for optimum cultural practices. For example, 2,4-D will not overcome the size problem resulting from an inadequate supply of moisture to trees (Erickson and Richards, 1955).

A significant reduction in splitting at the navel end of Washington navel oranges also has resulted from 2,4-D treatments applied when fruits are small. In addition, the onset of granulation has been delayed in Valencia oranges by applying 2,4-D to small fruits.

IMPROVING FRUIT SET

A means of obtaining improved fruit set in citrus would prove of enormous commercial benefit, and plant-growth regulators may eventually provide the means of bringing about such responses. No appreciable success has been achieved thus far in improving fruit set of citrus with these compounds, but research results have been promising on a wide range of other plants.

Although delayed abscission of flowers and young fruits has resulted from 2,4-D applications to orange trees (Stewart and Klotz, 1947; Stewart *et al.*, 1951), the end result was a decrease in number of fruits reaching maturity. Such a thinning effect has been observed many times in California and on Valencia oranges in South Africa. A notable exception has been reported in South Africa, where number of fruits retained by navel orange trees were increased by 2,4-D (DeVilliers, 1955). Data from a number of experiments leave no doubt that increased volume of navel oranges from 2,4-D-treated trees in South Africa is both the result of increased numbers of fruit and increased fruit size. The reason for the response is not known, although climatic and soil moisture conditions prevailing during the flowering and fruit set period for navels in South Africa appear to be more adverse than in California.

Naphthaleneacetic acid (NAA), indoleacetic acid (IAA), and indolebutyric acid (IBA) failed to increase fruit retention in Washington navel orange and Marsh grapefruit (Pomeroy and Aldrich, 1943). Many other compounds have been tested in preliminary experiments, and all except gibberellic acid (GA) have failed to give the desired results.

In California, application of GA to small Bearss lime fruits (⅜-inch or 9.5-mm diameter), Eureka lemon flowers, and branches of Washington navel orange trees during flowering resulted in an increase in the number of fruits (Hield, Coggins, and Garber, 1958). No influence was detected on shape, size, color, or fruit quality. In navel oranges, an increase in small seedlike structures occurred which is similar to the effect caused by applications of 2,4-D to small fruits. In Japan, improved retention of navel orange fruits was reported when GA was applied by hand to individual organs during the flowering or young fruit period (Iwasaki *et al.*, 1962). Set of Clementine mandarin, a self-incompatible variety, was induced by GA application to flowers when pollen was excluded (Soost, 1958). Compared with Clementine fruits from pollinated flowers, treated fruits were smaller, more necked, elongated, and seedless. Increased fruiting was obtained when shoots and flowers of Orlando tangelo, Dream navel orange, and Valencia orange were dipped in GA solutions (Krezdorn and Cohen, 1962). With the Orlando tangelo, the number of fruits on GA-treated shoots was about equal to that obtained with Temple tangor crosses and superior to that obtained with open-pollinated shoots. Slight fruit malformations and splitting occurred with Dream navel and Valencia orange. Increased fruit retention was observed when Pineapple orange shoots with small fruits were dipped in GA (Krezdorn and Cohen, 1962). Green fruits, thicker peels, and some splitting were associated with this treatment.

Because of the need for treatments that will increase fruit set, detailed experiments have been conducted in California with GA on navel orange, Valencia orange, and Clementine mandarin. After navel orange trees were sprayed with relatively high concentrations of GA during the flowering period, drastic injury and reduced production occurred (Hield, Coggins, and Garber, 1965). Injury was expressed in leaf drop and death of new vegetative and flower-producing shoots. GA concentrations low enough not to cause injury produced no increase in fruit set. Although considerable leaf drop occurred, an increase in fruit retention was obtained when Valencia orange trees were sprayed with high levels of GA (Coggins, Hield, and Garber, 1960). Treatments were applied when the trees were past full bloom, with small fruit of up to ⅜-inch diameter. Increased fruit set and production resulted from GA applications to low-yielding Clementine mandarin trees, but production was not improved under conditions of adequate cross-pollination (Soost and Burnett, 1961). Fruit produced by GA-treated Clementine mandarin trees were small and coloring was delayed. Delayed coloring and certain juice quality effects suggested that the treatment resulted in delayed maturity.

In Florida, studies have been conducted in which GA was sprayed on entire trees (Krezdorn and Cohen, 1962). Late bloom sprays of GA on Orlando tangelo increased yield and number of fruits. Leaf drop, fruit splitting, and coarse, green fruits borne on thick, stiff stems resulted in some cases. Many fruits were extremely small. Treated fruits remained green longer, but were well colored at harvest. More recently, Krezdorn (1968, unpublished) showed a substantial increase in fruit set on Orlando tangelo with applications of 2.5 to 10 ppm of GA during bloom. Only a slight reduction in fruit size occurred, and no other adverse effects were noted. No increased fruit set was obtained when Dream navel and Valencia orange were sprayed with GA (Krezdorn and Cohen, 1962).

In most cases, the potential advantage of improved fruit set through use of GA has been overshadowed by undesirable responses. Nevertheless, the fact that GA can increase fruit set in California and Florida, where other plant-growth regulators have failed, indicates a potential for the future.

BIENNIAL BEARING AND THINNING

With many perennial plants, the production of alternate heavy and light crops is a problem. One undesirable feature of the biennial production pattern is the inferior size of fruits during the heavy crop year. Naphthaleneacetic acid (NAA) has been widely employed as a thinning agent in apples and to some extent in peaches, pears, and olives. Reduction of the number of fruits leads to an increase in average size and also reduces biennial bearing.

Certain citrus varieties are especially prone to production of alternate heavy and light crops. Under California conditions, the Wilking mandarin exhibits an extreme cycle of a heavy crop during the "on" year followed by essentially no flowers nor crop during the "off" year. During heavy crop years, fruits are exceedingly small, and the total crop load is sometimes so heavy that major tree branches are broken. NAA sprays to several citrus varieties during bloom showed no influence on fruit set (Pomeroy and Aldrich, 1943; Stewart *et al.*, 1952), but applications to Wilking mandarin at about the normal period of "June drop" produced a thinning effect and improved fruit size (Hield, Burns, and Coggins, 1962). Valencia orange, navel orange, and a number of mandarin varieties have been thinned with NAA applied near the normal period of "June drop." At present, we are unable to obtain the proper amount of thinning with the compound, but it appears to have no undesirable influence on external or internal fruit quality. To have an appreciable influence on the alternate crop year, it appears that the thinning effect must be of sufficient magnitude to reduce the volume of fruit pro-

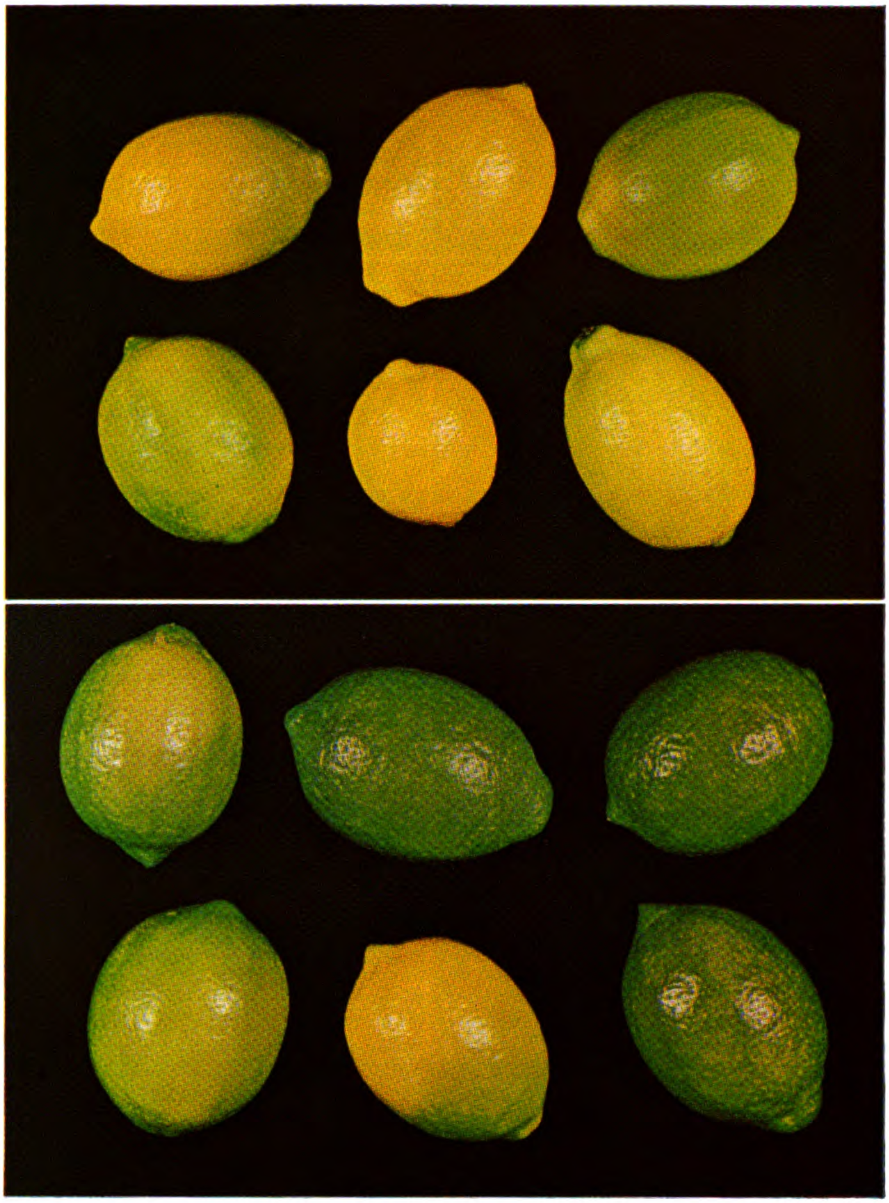

Fig. 6-2. Delay of lemon fruit maturation resulting from gibberellic acid treatments. Top, fruit from untreated trees. Bottom, fruit from treated trees. Treatment of fruit retards ripening. Because treated fruit remain green longer, fewer fruit develop the yellow color at a time when they are smaller than desirable for picking.

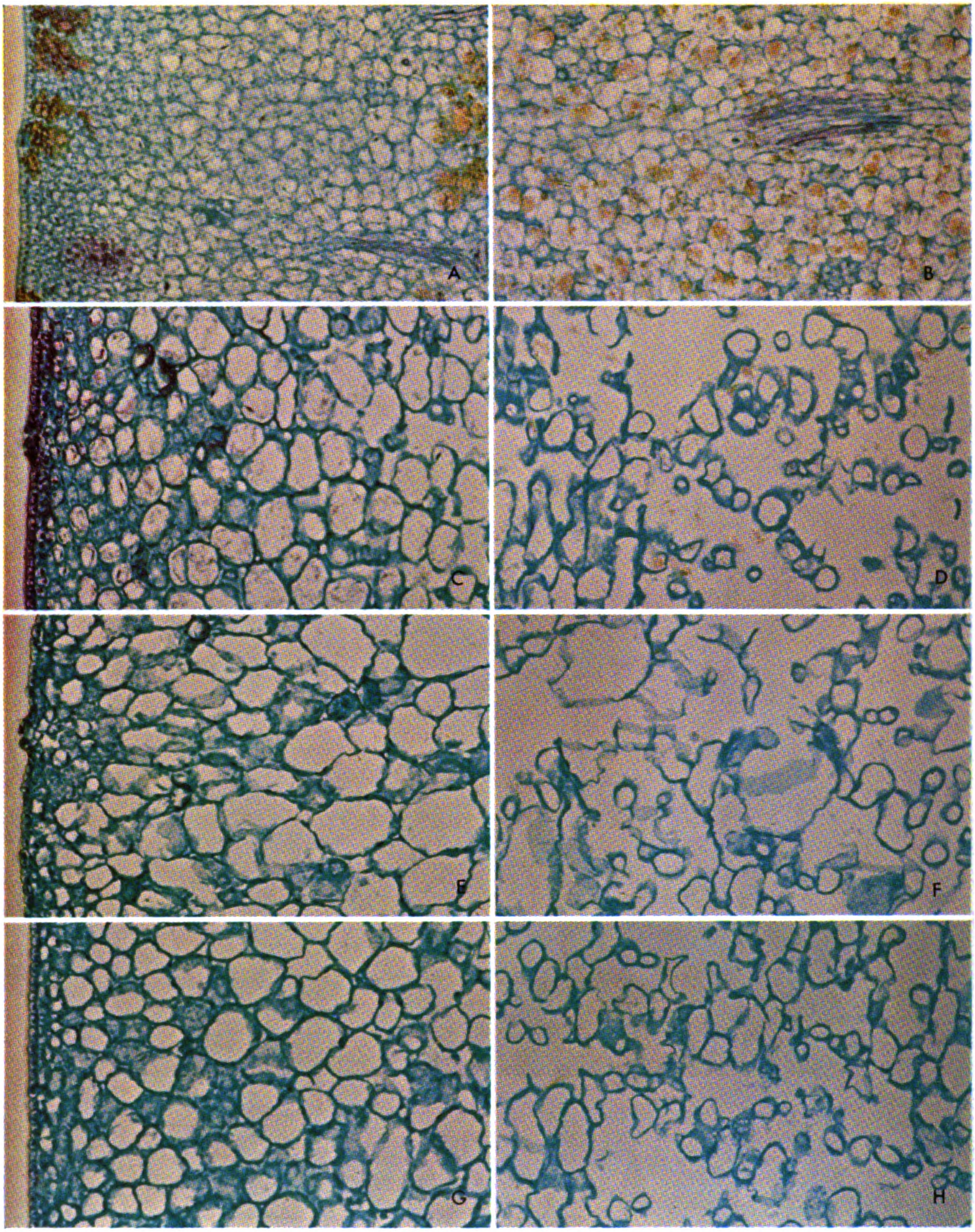

Fig. 6-3. Sections of pericarp of navel orange. *A*, *B*, flavedo and albedo, respectively, two months after anthesis. Note small, compact cells and large number of brown hesperidin crystals. *C*, *D*, flavedo and albedo eight months after anthesis. *E*, *F*, flavedo and albedo fifteen months after anthesis. Note large, elongated, highly vacuolated cells and the numbers of intercellular spaces. *G*, *H*, flavedo and albedo fifteen months after anthesis, following treatment with GA nine months earlier. Note relatively young appearance of tissue as evidenced by the compactness and integrity of cells. (A-H, X 440.)

Table 6–3

EFFECTS OF NAA FRUIT-THINNING SPRAYS ON THE WILKING MANDARIN IN COACHELLA VALLEY, CALIFORNIA

Factors	Date	Untreated Control	NAA Treatment (Ppm)		
			350	500	1,000
		Experiment 1			
Sprayed May 20, 1960:					
Relative fruit drop (no.)	June 2, 1960	0.4	28.1*	93.6**	....
Fruit diameter (mm)	Jan. 10, 1961	60.5	62.9*	66.0**	69.5**
Yield (boxes per tree)†	Feb. 14, 1961	3.2	3.1	2.4	1.4
Not sprayed, 1961:					
Fruit diameter (mm)	Jan. 3, 1962	69.8	67.7	72.3	55.9
Yield (boxes per tree)	Feb. 7, 1962	0.7	0.4	2.3*	2.3*
Yield (fruit per tree)	Feb. 7, 1962	97.6	50.3	382.5	459.6*
		Experiment 2			
Sprayed May 23, 1961:					
Relative fruit drop (no.)	May 29, 1961	12.2	9.8	18.5	....
Relative fruit drop (no.)	June 23, 1961	72.3	237.5**	266.7**	....
Fruit diameter (mm)	Jan. 10, 1962	53.6	61.7**	64.5**	....
Yield (boxes per tree)	Feb. 7, 1962	5.6	4.3**	3.8**	....
Yield (fruit per tree)	Feb. 7, 1962	1,392.8	782.5**	550.0**	....

Source: Hield *et al.* (1962).

* Indicates difference from the control at the 0.05 level of probability.

** Indicates difference from the control at the 0.01 level of probability.

† Field box internal dimensions: 24 by 15 by 7.5 inches (1.56 cubic feet or 0.044 cubic meters).

duced. With the resulting increase in fruit size, it is possible to greatly decrease the number of fruits without altering the volume produced by the tree. This is illustrated by the data on Wilking mandarin studies presented in table 6–3.

Studies in Florida indicated that maleic hydrazide (MH) is an effective blossom-thinning agent for Dancy tangerines (Gardner, Reece, and Horanic, 1961). No thinning was obtained when the compound was applied after petal fall. Unlike the results obtained with NAA, no increase in fruit size was associated with thinning. A reduction in fruit size, suppressed development of juice vesicles, and increased rind thickness resulted from applications of MH to Washington navel orange trees (Erickson *et al.*, 1952). No such fruit quality effects were reported on Dancy tangerines. Whether this reflects differences in susceptibility, differences in penetration, or an interaction with environmental factors is not known. Hendershott (1962*a*) reported that the rate of absorption of MH by Valencia orange leaves is faster in a relative humidity of 90 to 95 per cent than at 60 to 70 per cent.

FRUIT MATURITY

Plant-growth regulators are a potential means of regulating various aspects of fruit maturity. The development of methods for bringing about early fruit maturity or delaying fruit maturation could prove of considerable advantage for numerous citrus varieties grown under a broad range of climatic conditions and subject to various marketing demands.

It has been demonstrated that GA delays loss of green rind pigments by navel orange (Coggins and Hield, 1958), Valencia orange (Coggins, Hield, and Garber, 1960), grapefruit (Coggins, Hield, and Burns, 1962), tangerine (Soost and Burnett, 1961), lemon (Coggins, Hield, and Boswell, 1960), lime (Burns *et al.*, 1964), and satsuma mandarin (Nishiura and Iba, 1964). High concentrations of GA applied to Lisbon lemon trees in the spring caused a significant delay in fruit maturity as well as a number of undesirable responses. Production during the treatment year was reduced, apparently as a result of increased fruit drop. Fruit drop and the percentage of harvested lemons without buttons were increased. Fruit stems showed an increase in diameter, a response also reported for 2,4-D. After a very sparse bloom in treated plots, production was drastically reduced the next year. The desirable feature of the treatment, a delay in yellowing, was marked: lemons from treated trees were greener as early as one month and for as long as seven months

Table 6–4

INFLUENCE OF GIBBERELLIC ACID ON PERCENTAGE OF YELLOW LEMON FRUIT

GA Treatment† (Ppm)	Harvest Dates (1960) March	May	June	August	October
0	51.4	60.5	49.8	42.4	34.5
5	39.8**	44.7**	45.3	40.3	29.4
10	32.8**	38.0**	36.9**	36.6	30.6
20	25.8**	28.5**	28.7**	31.2*	25.1
40	17.2**	23.4**	22.2**	18.3**	25.0

* Statistically significant at the 0.05 level of probability.
** Statistically significant at the 0.01 level of probability.
† Color evaluated on the stored portion of the fruit. The GA treatment was applied on November 17, 1959.

after treatment. Fruits treated with GA also appeared to develop a lemon-yellow color less rapidly in storage. Ultimate fruit size was not reduced by GA, but fruits of 3.6 to 4.7 cm in diameter at treatment time grew more slowly than untreated fruits. Growth rate of fruits that were larger or smaller at treatment time appeared to be normal.

Delayed yellowing has been induced by GA applied to lemon trees at any time of the year. The response reflects an over-all delay in maturity rather than a simple delay in rind maturity as appears to be the case with navel orange. Very few undesirable responses, if any, occur when relatively low concentrations of GA are employed. The authors have found that the over-all influence of an appropriate dosage applied in the fall appears to be beneficial and of economic value. In addition to the first-year effects on fruit color (table 6–4 and fig. 6–2), fruit size (table 6–5), and seasonal harvest patterns (table 6–6), there is a change in harvest pattern in the second year (table 6–7). Probably this results from the influence of GA on flowering, which has been observed in numerous trials. The volume of fruit produced early in the season is reduced, but treated trees produce more fruit during summer when the market demand is high. If GA is applied in two successive years, the seasonal harvest pattern is altered even more, since both direct and indirect effects are operative (table 6–7).

Table 6–5

INFLUENCE OF GIBBERELLIC ACID ON SMALL LEMON FRUIT PLACED IN STORAGE

(Percentage of fruit smaller than 2.01 inches or 5.1 cm in diameter)

GA Treatment† (Ppm)	Harvest Dates (1960) May	June	August	October
0	15.4	12.4	12.1	32.8
5	5.8**	6.7**	6.5**	32.2
10	4.5**	4.8**	5.7**	28.2
20	3.4**	4.4**	3.6**	29.4
40	2.7**	3.5**	3.8**	21.2

* Statistically significant at the 0.05 level of probability.
** Statistically significant at the 0.01 level of probability.
† GA treatments applied on Nov. 17, 1959.

The primary benefits of GA treatment are a more desirable seasonal harvest pattern in relation to market demands, a larger percentage of fruit with a long storage life, and a decrease in the number of small yellow lemons. These effects permit more flexibility in harvesting and marketing. Since the California lemon industry is based on the sale of fresh fruit and peak production normally occurs when demand is low, these findings have important economic implications. Gibberellic acid is registered (see p. 386) and recommended for use on lemons in California (Coggins *et al.*, 1964). Similar responses have been obtained from GA treatment of limes (Burns *et al.*, 1964).

Erickson and Haas (1956) showed that some delay in fruit maturity results from application of 2,4,5-T to lemon trees, although it appears to be minor compared to that produced by GA.

Gibberellic acid treatments are recommended also for the navel orange in California. As the navel orange approaches legal maturity in California, the concentrations of chlorophyll pigments in the flavedo of the fruit decrease and carotenoid pigments increase. During the change in color from green to orange, the rind softens rapidly for a period and then continues softening at a slower rate throughout the harvest season (Coggins and Lewis, 1965). Since color development commences prior to the attainment of legal maturity, softening actually begins before the start of the harvest season, continuing for as long as fruit remain on the tree. Under California cultural and marketing conditions, fruit may be harvested as late as eight months after onset of softening. Thus, there is ample opportunity for extremely soft rind tissue to develop, along with other senescence effects, contributing to a number of physiological rind disorders in the late harvest season which reduce shelf life and market value.

During the eight-month softening period in California, the capacity of rind tissue to utilize glucose decreases, sugars accumulate to approximately twice their initial concentration, and the

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Table 6–6

INFLUENCE OF GIBBERELLIC ACID ON LEMON TREE HARVEST PATTERNS ONE YEAR AFTER TREATMENT

(Percentage of Crop Harvested at Specified Times)

GA Treatment†	Harvest Dates (1960–61)					
(Ppm)	March	May	June	August	October	January
0	9.2	46.5	23.7	12.3	5.8	2.5
5	10.0	40.6**	24.1	16.9*	5.8	2.6
10	10.1	39.4**	23.7	17.0*	7.2**	2.6
20	11.2	37.7**	22.7	17.9**	8.0**	2.5
40	10.3	36.2**	22.8	18.6**	9.5**	2.6

* Statistically significant at the 0.05 level of probability.

** Statistically significant at the 0.01 level of probability.

† GA treatments applied on Nov. 17, 1959.

Table 6–7

INFLUENCE OF GIBBERELLIC ACID ON LEMON TREE HARVEST PATTERNS

(Percentage of Crop Harvested at Specified Times)

GA Treatment (Ppm)†		Harvest Dates (1961)			
1959	1960	May	July	September	November
0	0	39.7	26.1	15.9	18.3
0	0	41.1	26.1	15.3	17.5
5	0	31.0	28.1	17.6	23.3
5	5	28.2	31.7	18.1	22.0
10	0	32.2	29.1	18.5	20.2
10	10	26.4	31.2	19.0	23.4
20	0	26.7	32.4	20.0	20.9
20	20	20.9	28.1	23.2	27.8
40	0	17.5	33.6	22.9	26.0
40	40	17.8	28.2	24.0	30.0
Concentrations		***	**	***	***
Times		**	N.S.	N.S.	***
Times × concentrations		**	***	N.S.	***

** Statistically significant at the 0.01 level of probability.

*** Statistically significant at the 0.001 level of probability.

N.S. = non-significant at the 0.05 level of probability.

† GA treatments applied in November, 1959, and November, 1960.

ratio of potassium to calcium plus magnesium increases (Lewis *et al.*, 1967). These physiological changes suggest that mitochondrial and plasmalemma membrane systems become less functional during aging.

Recent histological studies by the senior author provide a structural basis for the soft condition that exists in senescent rind. A detailed description of rind tissue is presented in Chapter 1, page 17. Interested readers also should refer to studies of navel and Valencia orange rinds reported by Scott and Baker (1947) and Bain (1958). These studies provide excellent descriptions of the structural changes that take place from anthesis to maturity, although the senescent stage is not considered in detail. In general, cells of the flavedo and albedo enlarge, become highly vacuolated, and change shape during development, maturation, and senescence (fig. 6–3, *A–F*; magnifications are expressed on an area basis). Many intercellular spaces develop, and cell walls appear to weaken and break, particularly in the albedo. Tissue of the senescent rind appears to be structurally weak, and the relatively low amount of cytoplasm suggests that the tissue may possess low metabolic activity.

Preharvest treatment of individual navel orange fruits at the commencement of softening with 500 ppm of GA causes a considerable delay in the rind maturation and senescence processes (fig. 6–3, *G–H*). Although the response to high concentration treatments of individual fruits is greater than that obtained with entire tree sprays of 5 to 20 ppm of GA, the same types of anatomical changes probably account for the presence of firmer rinds on fruits treated with recommended rates.

The events leading to senescence in navel orange rind appear to include pigment changes, softening of the tissue, increases in carbohydrates, some changes in carbohydrate metabolism, and changes in cation concentrations. All of these factors clearly are modified by GA, resulting in a delay in senescence.

Rind disorders that appear to be associated with aging include rind staining, water spot, increased susceptibility to decay, puffy rinds, and accumulation of a rind exudate. Each of these disorders can be reduced by GA treatments.

The reduced susceptibility of GA-treated fruits to rind staining (Eaks and Jones, 1959; Coggins *et al.*, 1963) is probably related to the retardation of the softening process. Rind staining is a physiological disorder associated with mechanical abrasion during harvesting, handling, and packing. Treatment with GA to reduce rind staining also delays loss of green pigments. The delay in loss of pigment can be a serious disadvantage, if conditions make it necessary to harvest while the fruits still retain chlorophyll, since consumers interpret this characteristic as a sign of immaturity. Treatment should be carried out after a marketable color has developed.

When navel oranges age on the tree, the rind surfaces occasionally become sticky. The sticky material cannot be removed by washing and packing operations, and thus constitutes a problem that detracts from quality. The condition has been substantially reduced by preharvest treatments with GA (Coggins *et al.*, 1965).

Various oil emulsion formulations sprayed on the foliage of navel orange trees in late summer or fall to control scales, mites, and other citrus pests increase the susceptibility of fruit to water spot. This disorder is brought about by surface deposits of water entering the rind and accumulating in its tissues. The rind spots serve as ready points of entry for decay organisms. Even those fruits that escape decay are of no value on the fresh fruit market. Treatment of fruit with GA appears to substantially reduce water spot (Riehl, Coggins, and Carman, 1965).

Although delayed aging of grapefruit and Valencia orange rind tissues is a useful response, treatment of these fruits with GA results in two rind color problems. Gibberellic acid applied to green fruits delays loss of green pigments; applied to fruit of mature color, it enhances regreening.

FRUIT STORAGE

The keeping quality of fruit is a primary concern of the citrus industry. In general, the maximum interval between harvest and consumption of oranges and grapefruit is two months. Lemons may be kept in lengthy storage before being placed in cartons for shipment. Length of storage of lemons ranges from a few weeks for yellow-colored fruit to five or six months for dark-green fruit. Extensive research is being conducted on means of prolonging storage and reducing storage disorders.

Prior to the late 1940's, Alternaria decay was one of the most serious fungus diseases of stored lemons. The first evidence that Alternaria decay could be controlled by modification of fruit physiology came from preharvest treatments with 2,4-D and 2,4,5-T. In both preharvest and postharvest treatments, 2,4,5-T has proven more effective in control of the disease than 2,4-D, and ester formulations have been more effective than amine salt formulations. Table 6–8 presents data comparing the effects of 2,4-D and 2,4,5-T on storage life of lemons. Since no residue tolerance has been established for 2,4,5-T, however, this discussion is centered around the use of 2,4-D.

Although 2,4-D is relatively ineffective in inhibiting the growth of Alternaria *in vitro* (Erickson, DeWolfe, and Brannaman, 1958), treatment of fruit with this compound prior to storage reduces the fungus to a minor role in storage decay (Stewart, 1949; Stewart, Palmer, and Hield, 1952; Erickson, 1952; Iwasaki, Nishiura, and Shichijo, 1956). The primary reason for success appears to be that treated lemons are more resistant to the entry of fungus. Once the fungus has gained entrance, it grows as well in treated as in nontreated fruits (DeWolfe, Erickson, and Brannaman, 1959). Alternaria spores are present under the button (receptacle and calyx) of most lemons (Bartholomew, 1926). Once the button dies, invasion can readily occur. Treatment with 2,4-D delays the development of the abscission layer, the button remains in a living condition (table 6–8), and entry of the fungus is reduced. The usual practice is to apply 2,4-D as a final step in the washing procedure for lemons. Where wax is applied prior to

Table 6–8

EFFECT OF 2,4-D AND 2,4,5-T ON STORAGE OF YELLOW, SILVER, AND LIGHT-GREEN LEMON FRUITS

Treatment	Months of Storage	Per Cent Black Buttons			Per Cent Alternaria Decay			Per Cent Miscellaneous Decay		
		Yellow	Silver	Light-Green	Yellow	Silver	Light-Green	Yellow	Silver	Light-Green
Wax emulsion, no growth regulator	2	....	5.4	....	....	0.5	....	....	0.5	....
	4	....	58.5	44.0	....	2.7	0.0	....	1.9	3.7
	6	....	73.2	64.0	....	9.5	5.2	....	14.5	10.5
Wax emulsion, 500 ppm of 2,4-D*	2	0.4	0.0	....	0.0	0.0	....	0.0	0.0	....
	4	2.9	1.1	0.0	0.5	0.0	0.0	0.4	1.1	1.1
	6	2.5	0.0	0.0	1.5	0.1	0.0	2.7	0.0	1.7
Wax emulsion, 100 ppm of 2,4,5-T†	2	0.0	0.0	....	0.0	0.0	....	1.8	0.0	....
	4	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0
	3	0.9	0.0	0.7	0.0	0.5	0.0	0.9	1.1	0.0
Wax emulsion, 200 ppm of 2,4,5-T†	2	0.0	0.0	....	0.5	0.0	....	1.5	3.2	....
	4	0.0	0.0	0.0	0.5	0.0	0.0	2.0	0.0	0.5
	6	0.0	0.6	0.0	0.5	0.6	0.0	2.0	0.0	0.0
Wax emulsion, 500 ppm of 2,4,5-T†	2	0.5	0.0	....	0.5	0.0	....	0.5	0.0	....
	4	0.5	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.5
	6	0.0	0.0	0.0	0.5	0.0	0.0	2.9	0.0	0.6

Source: Unpublished data from the files of the junior author and Louis C. Erickson.

* Isopropyl ester.

† Amyl and isopropyl ester mixture.

storage, 2,4-D is added with the wax.

Treatment with 2,4-D also delays aging and reduces external decay (which is probably a result of retardation of the aging process). Delayed aging is characterized by firmer fruits and a delay in development of the lemon-yellow color. If the dosage is too high, the delay in coloring can become a problem.

A reduction in black buttons, reduced Alternaria decay, and delayed aging also has resulted from the use of 2,4-D on oranges and grapefruits (Stewart, 1949). Delayed yellowing of Persian limes (Hatton, 1958) and improved storage life of Clementine mandarin (Gutter, 1956) has been effected by postharvest treatments with 2,4,5-T.

FLOWERING

There is little doubt that flowering of plants is under chemical control, since qualitative and quantitative changes of plant-growth substances occur during flower induction and development (Harada and Nitsch, 1959). However, which changes are the cause and which are the result of flowering has not been established. Among the major environmental factors known to be critical for flowering of most plants are temperature, soil moisture, and photoperiod. The precise effects and interactions of these and other factors on flowering are not known in detail. In the case of citrus, it is clear that flowering usually follows the breaking of dormancy induced by an appreciable period of cool temperatures or drought. Photoperiod does not appear to significantly influence the flowering of citrus (Furr, Cooper, and Reece, 1947).

Methods to accelerate and delay flowering could be of considerable advantage in citrus breeding and commercial production. Breeding programs and early production, especially in close plantings, would benefit from the ability to cause young trees to flower and set fruit. Very young juvenile citrus seedlings, especially grapefruit, often produce a single terminal flower, and then the plant normally does not flower again until it has passed through the juvenile phase of growth (Furr *et al.*, 1947). Flower induction of young grapefruit seedlings appears to be favored by certain low-temperature situations (Hield, Coggins, and Lewis, 1966), although these same conditions do not appear to induce flowering in older seedlings.

Experiments directed toward the control

of the flowering process have shown promise for the future. Kessler, Bak, and Cohen (1959) found that xanthine, uracil, and 2,3,5-triiodobenzoic acid promoted the flowering of young grapefruit seedlings, but did not affect four-year-old trees. Monselise and Halevy (1964) obtained flower induction on branches of young lemon trees from applications of benzothiazole-2-oxyacetate. Some slight effect was obtained from (2-chloroethyl)trimethylammonium chloride and *N*-dimethylamino succinamic acid. A pronounced decrease in vegetative growth and a marked increase in numbers of flowers produced the following spring resulted from the application of *N*-dimethylamino succinamic acid to apple, pear, and sweet cherry trees (Batjer, Williams, and Martin, 1964).

Monselise and Halevy (1964) inhibited flowering of orange trees with high concentrations of GA. Delayed flowering of lemons also has been observed after GA treatments. To bring harvest period into phase with demand, delayed flowering would prove particularly advantageous with lemons and limes.

Both delayed flowering and inhibition of flowering after GA treatment have been reported for a number of tree crops (Hull and Lewis, 1959; Crane, Primer, and Campbell, 1960; Griggs and Iwakiri, 1961). A combination of 2,4-D and NAA has delayed the flowering of cherry trees (Tukey and Hamner, 1949). Such results usually have been accompanied by undesirable vegetative effects.

VEGETATIVE EFFECTS

Some information is available on the influence of plant-growth substances on the vegetative growth of citrus. One vegetative growth response, the use of maleic hydrazide (MH) to enhance dormancy and reduce susceptibility to frost, is already being used commercially to some extent in Florida.

Haas and Brusca (1954*a*) improved the germination of Koethen sweet orange seeds by soaking them in dilute solutions of 2,4-D and by applying the solution to seed beds. The same authors (1954*b*) studied the effect of soil applications of 2,4-D on growth of eight citrus varieties. Certain varieties were stimulated to greater vertical growth at low concentrations of 2,4-D, whereas higher levels increased foliage density rather than height.

Table 6–9

EFFECT OF MALEIC HYDRAZIDE (MH) SPRAYS ON SHOOT REGROWTH IN TOP-PRUNED LEMON TREES

MH Treatment (Ppm)	Mean Regrowth in Feet†	
	Sept. 26, 1961**	Mar. 15, 1962*
0	5.1_{w}	6.1_{w}
100	3.8_{x}	4.6_{wx}
200	3.3_{x}	4.8_{wx}
300	3.2_{xy}	4.6_{wx}
400	2.6_{yz}	3.4_{xy}
500	2.5_{yz}	3.8_{xy}
600	2.4_{yz}	3.4_{xy}
700	2.5_{yz}	3.7_{xy}
800	2.2_{yz}	3.7_{xy}
900	2.0_{z}	3.2_{xy}
1,000	1.9_{z}	3.1_{xy}

Source: After Hield *et al.* (1963).

** Statistically significant at 0.01 level of probability.

* Statistically significant at 0.05 level of probability.

† Sprayed May 25, 1961. Means followed by common subscript letter are not significantly different from each other. No statistical evaluations have been made of the values between columns.

Increased seedling growth has been obtained with GA (Marth, Audia, and Mitchell, 1956; Lange, 1957; Kuykendall, 1958; Randhawa and Singh, 1959; Monselise and Halevy, 1962; Nishiura and Iba, 1964), although the same treatment was found by some of the investigators to decrease root growth (Kuykendall, 1958; Monselise and Halevy, 1962; Nishiura and Iba, 1964). Marth *et al.* (1956) reported an initial increase in shoot growth rate after treatment with GA, but no effect on height of plants was evident four months after treatment.

In Texas, Cooper and Peynado (1958) treated young grapefruit trees with GA, initiating shoot growth during periods between the spring, summer, and winter growth flushes. As with untreated trees, growth on treated trees occurred in a series of flushes and total growth was not improved by GA. Some treated shoots developed long thorns and abnormally narrow leaves. Essentially the same results have been obtained with young navel trees in California.

A common cultural practice with lemons and some other citrus trees is removal of part of the tops of the foliage canopy. Frequent top mowing is a practice in many lemon groves. Hield, Coggins, and Boswell (1963) demonstrated that application of MH to young regrowth in the top of lemon trees will suppress subsequent growth (table 6–9), but have little effect on the rest of the tree. Growth inhibitors such as MH may eventually prove useful in suppressing top growth, restricting lateral growth in close plantings, and maintaining a desirable tree shape for mechanical harvesting.

Dormant citrus is more frost resistant than actively growing trees (Cooper, 1959). Prolonged dormancy has been obtained by winter application of MH to Valencia orange and grapefruit trees in California (Erickson *et al.*, 1952). Although MH has been shown to be generally ineffective for inducing dormancy of trees in Texas (Cooper, Peynado, and Otey, 1955; Cooper, 1959), it has shown promise in Florida (Stewart and Leonard, 1960). Treatment of nonbearing Florida trees induced dormancy and increased frost resistance. The length of dormancy varied from one month to as long as six months after sprays of 1,000 ppm of MH were applied in two consecutive years (Hendershott, 1962*b*). In California, where cooler winters prevail, studies indicated that MH is of uncertain value for increasing the frost tolerance of young trees (Burns, Garber, and Hield, 1962). The fact that GA can be employed to break natural dormancy in citrus (Cooper and Peynado, 1958) raises the question of whether GA can be used to break MH-induced dormancy. A number of other compounds have failed to induce dormancy in citrus (Cooper, 1959).

Plant-growth substances have played a major role in enhancing the rooting of cuttings from a wide range of plants. As early as 1935, Cooper (1935) reported that lanolin containing IAA greatly increased the rooting of lemon cuttings. Treatments not only caused increased production of roots on leaf-bearing cuttings, but also initiated root formation on leafless cuttings. No roots formed on the untreated leafless cuttings. Cooper (1938) reported that treated lemon cuttings produced a band of meristematic cells between the phloem and xylem in two days. Within six days, some of these cells had differentiated into root primordia.

Cooper (1940) found that citrus appeared to root best from leaf-bearing, mature terminal growth. IAA and indolebutyric acid (IBA) treatments seemed to be equally effective in enhancing the rooting of many citrus varieties, but treatment with talc dust containing the compounds was not as effective as soaking the base of cuttings for twenty-four hours in water solutions of the growth regulators.

Although IAA and IBA have proven equally effective in promoting rooting of many citrus varieties, NAA was reported superior to IAA for the rooting of sweet lime (Jauhari and Rahman, 1958). Kulkarni (1957) showed that indolepropionic acid could be used to improve the rooting of lemon cuttings. Erickson and Bitters (1953) investigated the effect of NAA, IBA, 2,4-D, and 2,4,5-T on the rooting of one representative each of five Rutaceae genera other than *Citrus*. No single compound that enhanced rooting was found best for all of the genera studied.

Improved bud grafting in grapefruit has been reported with IAA and IBA. Removal of wood from the bud shield combined with IBA gave the best results (Maiti, Singh, and Singh, 1959).

RESIDUES OF PLANT-GROWTH REGULATORS

The possibility of residues of plant-growth regulators remaining in citrus fruit at harvest presents a problem that will become increasingly important as commercial applications are developed for these compounds. In the United States, it is illegal for fruits to enter into interstate commerce unless a product used in treatment has been registered with the U.S. Department of Agriculture and the Food and Drug Administration has established a safe residue tolerance for the compound.

Before any registration of the product can be approved, there must be evidence of a cultural problem of sufficient magnitude to justify the use of an agricultural chemical to combat it. Such a chemical must provide some control over the problem and be relatively safe for the crop in question as well as the production of subsequent crops. The chemical employed in treatments must leave no toxic residue in the crop, or any residue must be of such low magnitude as to present no health hazard.

With the exception of 2,4-D and gibberellic acid, little data is available concerning residues of plant-growth regulators in citrus tissues. By means of bioassay tests, Stewart *et al.* (1952) found rind surface residues of 2,4,5-T in citrus fruits two and one half months after postharvest application of triethanolamine salt. Their studies revealed no residues from postharvest applications of the isopropyl or butyl esters of 2,4,5-T. Erickson (1955) calculated that the deposit left by postharvest applications of 2,4-D to lemons averaged between 1.8 and 2.8 ppm of the fruit weight. More recently, Erickson and Hield (1962) described a method of analysis for 2,4-D in citrus fruits and reported residue data obtained. They found that oranges from trees sprayed with 20 ppm of 2,4-D contained an average of 0.1 ppm of the compound one day after treatment and that postharvest applications of 500 ppm to lemons gave an average of 1.1 ppm two days later. One week after treatment, the oranges contained less than 0.1 ppm of 2,4-D. Most of the 2,4-D was recovered as acid, although some unknown ester-like residue was also present. Since the isopropyl ester of 2,4-D was applied, there was an unresolved question as to whether this particular molecule was present as part of the residue. Evidence is now available that all of the isopropyl ester entering the fruit is hydrolyzed and is not found as residue. Either 2,4-D acid or the isopropyl ester can serve equally well for the 2,4-D portion of the unknown ester-like compound that is synthesized by the plant tissues (Erickson, Brannaman, and Coggins, 1963).

Two plant-growth regulators are presently registered for use in treatment of citrus in the United States. Preharvest sprays of the isopropyl ester of 2,4-D are registered for oranges, grapefruit, and lemons. Postharvest sprays of 2,4-D on lemons also are registered. Gibberellic acid is registered for preharvest use on fruiting lemon and navel orange trees. Because no significant residues were detected when gibberellic acid was employed in preharvest treatments for the purpose of delaying fruit maturity and rind senescence, registration for its use in California was obtained on a no-residue basis. The registration stipulated that lemon fruits may not be harvested within thirty days and navel oranges within ten days after application.

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